WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, July 19, 2006

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT; PLUR=YES; OP=OR		
☐	L19	fold$.clm. and l16	3
☐	L18	periplasm$.clm. and l16	1
☐	L17	periplasm$ and l16	15
☐	L16	Pluckthun.in. or plueckthu.in.	20
☐	L15	((peptidyl adj4 prolyl adj4 isomerase) or PPI) same ((heterologous or antibody) same fold$)	11
☐	L14	(peptidyl adj prolyl adj isomerase) and ((heterologous or antibody) same fold$)	81
☐	L13	(peptidyl adj prolyl adj isomerase) and (heterologous or antibody) same fold$	81
☐	L12	(peptidyl adj prolyl adj isomerase) same (heterologous or antibody)	15
☐	L11	(peptidyl adj prolyl adj isomerase)	509
☐	L10	((peptidyl adj prolyl adj isomerase) or PPI)	5866
☐	L9	periplasm$ same (chaperon$ and fold$) same (heterologous or antibody)	18
	DB=USPT; PLUR=YES; OP=OR		
☐	L8	L7	154
	DB=PGPB,USPT; PLUR=YES; OP=OR		
☐	L7	periplasm$ same (chaperon$ or fold$) same (heterologous or antibody)	363
☐	L6	6027888.pn.	1
☐	L5	system and l1	1
☐	L4	fragment and l1	1
☐	L3	5246838.pn.	1
☐	L2	periplasmic and L1	1
☐	L1	6630317.pn.	1

END OF SEARCH HISTORY

PALM: 7/19/2006

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, July 19, 2006

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT; PLUR=YES; OP=OR		
☐	L15	((peptidyl adj4 prolyl adj4 isomerase) or PPI) same ((heterologous or antibody) same fold$)	11
☐	L14	(peptidyl adj prolyl adj isomerase) and ((heterologous or antibody) same fold$)	81
☐	L13	(peptidyl adj prolyl adj isomerase) and (heterologous or antibody) same fold$	81
☐	L12	(peptidyl adj prolyl adj isomerase) same (heterologous or antibody)	15
☐	L11	(peptidyl adj prolyl adj isomerase)	509
☐	L10	((peptidyl adj prolyl adj isomerase) or PPI)	5866
☐	L9	periplasm$ same (chaperon$ and fold$) same (heterologous or antibody)	18
	DB=USPT; PLUR=YES; OP=OR		
☐	L8	L7	154
	DB=PGPB,USPT; PLUR=YES; OP=OR		
☐	L7	periplasm$ same (chaperon$ or fold$) same (heterologous or antibody)	363
☐	L6	6027888.pn.	1
☐	L5	system and l1	1
☐	L4	fragment and l1	1
☐	L3	5246838.pn.	1
☐	L2	periplasmic and L1	1
☐	L1	6630317.pn.	1

END OF SEARCH HISTORY

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NEWS	6	MAY 11	KOREAPAT updates resume
NEWS	7	MAY 19	Derwent World Patents Index to be reloaded and enhanced
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NEWS	9	MAY 30	The F-Term thesaurus is now available in CA/CAplus
NEWS	10	JUN 02	The first reclassification of IPC codes now complete in INPADOC
NEWS	11	JUN 26	TULSA/TULSA2 reloaded and enhanced with new search and and display fields
NEWS	12	JUN 28	Price changes in full-text patent databases EPFULL and PCTFULL
NEWS	13	JUl 11	CHEMSAFE reloaded and enhanced
NEWS	14	JUl 14	FSTA enhanced with Japanese patents
NEWS	15	JUl 19	Coverage of Research Disclosure reinstated in DWPI

NEWS EXPRESS	JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
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NEWS X25	X.25 communication option no longer available

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=> fil medline biosis caplus scisearch embase wpids

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FULL ESTIMATED COST	0.21	0.21

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=> periplasm? and chaperon and fold and (heterologous or antibody)
MISSING TERM BEFORE 'AND'
Search expressions cannot begin with operators.

=> periplasm? and chaperon? and fold? and (heterologous or antibody)
L1          95 PERIPLASM? AND CHAPERON? AND FOLD? AND (HETEROLOGOUS OR ANTIBODY
               )

=> skp and l1
L2          18 SKP AND L1

=> fkp? and l1
L3          22 FKP? AND L1

=> l2 not l3
L4          12 L2 NOT L3

=> dup rem l1
PROCESSING COMPLETED FOR L1
L5          34 DUP REM L1 (61 DUPLICATES REMOVED)

=> dup rem l2
PROCESSING COMPLETED FOR L2
L6           7 DUP REM L2 (11 DUPLICATES REMOVED)

=> dupr rem l3
MISSING OPERATOR REM L3
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> dup rem l3
PROCESSING COMPLETED FOR L3
L7           6 DUP REM L3 (16 DUPLICATES REMOVED)

=> d ibib abs l6 1-7

L6   ANSWER 1 OF 7     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2005651710     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 16198365
TITLE:              High-level bacterial secretion of single-chain alphabeta
                    T-cell receptors.
AUTHOR:             Maynard Jennifer; Adams Erin J; Krogsgaard Michelle;
                    Petersson Karin; Liu Corey W; Garcia K Christopher
```

CORPORATE SOURCE: Department of Microbiology and Immunology and Howard Hughes Medical Institute, Stanford University, Stanford, CA 94304, USA.. jmaynard@umn.edu
CONTRACT NUMBER: F32 AI055245-01 (NIAID)
NIH-AI8540 (NIAID)
SOURCE: Journal of immunological methods, (2005 Nov 30) Vol. 306, No. 1-2, pp. 51-67. Electronic Publication: 2005-09-12. Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200602
ENTRY DATE: Entered STN: 16 Dec 2005
Last Updated on STN: 28 Feb 2006
Entered Medline: 23 Feb 2006

AB While numerous antibody-antigen systems have been structurally characterized, studies of structurally analogous T-cell receptor MHC systems have lagged behind largely due to the lack of a general TCR expression system. Efforts to develop bacterial systems have resulted in low yields (< 0.5 mg/l) of active material which is prone to proteolysis and aggregation. Here we report a strategy to secrete folded, soluble single chain T-cell receptors (scTCR) in the Escherichia coli periplasm using three representative alphabeta TCRs (172.10, 1934.4/c19 and 2B4). Shake flask yields between 0.5 and 30 mg/l active, purified material were attained for all TCRs studied and found to depend on the introduction of solubility-increasing amino acid substitutions, skp chaperone co-expression and C-terminal fusion to a human kappa constant domain in the context of a tightly regulated expression vector. This system will greatly enable crystallographic, thermodynamic and other biophysical analyses of TCRs which require large quantities of homogeneous material.

L6 ANSWER 2 OF 7 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:272937 SCISEARCH
THE GENUINE ARTICLE: 802HQ
TITLE: The periplasmic E-coli chaperone Skp is a trimer in solution: biophysical and preliminary crystallographic characterization
AUTHOR: Schlapschy M; Dommel M K; Hadian K; Fogarasi M; Korndorfer I P; Skerra A (Reprint)
CORPORATE SOURCE: Tech Univ Munich, Lehrstuhl Biol Chem, D-85350 Freising Weihenstephan, Germany (Reprint)
COUNTRY OF AUTHOR: Germany
SOURCE: BIOLOGICAL CHEMISTRY, (FEB 2004) Vol. 385, No. 2, pp. 137-143.
ISSN: 1431-6730.
PUBLISHER: WALTER DE GRUYTER & CO, GENTHINER STRASSE 13, D-10785 BERLIN, GERMANY.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 31
ENTRY DATE: Entered STN: 2 Apr 2004
Last Updated on STN: 2 Apr 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The 'seventeen kilodalton protein' Skp confers transient solubility on outer membrane proteins during biogenesis in Gramnegative bacteria. Here we report a first biophysical characterization of this chaperone itself, which also possesses biotechnological potential in the production of recombinant proteins. Using crosslinking and gel filtration methods, we found that Skp forms a stable homotrimer

in solution. Following thermal denaturation, monitored by CD spectroscopy, this chaperone refolds with high efficiency but exhibits a pronounced hysteresis between the un and refolding transitions. Using the recombinant protein equipped with the Streptag II at its Nterminus, suitable crystallization conditions for Skp were found. A first data set was collected to 2.60 Angstrom resolution.

L6 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:42454 CAPLUS
DOCUMENT NUMBER: 138:101974
TITLE: Method for improved recombinant protein expression in bacteria by monitoring and modulating protein folding using a degP promoter-based reporter system and coexpression of the chaperone Skp
INVENTOR(S): Riesenberg, Dieter; Horn, Uwe; Strittmatter, Wolfgang
PATENT ASSIGNEE(S): Merck Patent G.m.b.H., Germany; Riesenberg, Ulrike
SOURCE: PCT Int. Appl., 27 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003004698	A2	20030116	WO 2002-EP7346	20020703
WO 2003004698	A3	20031030		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2452849	AA	20030116	CA 2002-2452849	20020703
EP 1407052	A2	20040414	EP 2002-758299	20020703
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
JP 2004533261	T2	20041104	JP 2003-510456	20020703
US 2004185528	A1	20040923	US 2004-482728	20040105
PRIORITY APPLN. INFO.:			EP 2001-116371 A	20010706
			WO 2002-EP7346 W	20020703

AB This invention relates to a new method for improving functional protein expression whereby the folding process is monitored by online measurement and, if required, the protein folding is influenced by adding folding promoting agents and/or co-expression of the periplasmic chaperone (Skp). The accumulation of unfolded or misfolded protein in the periplasm of Escherichia coli leads to the induction of the well known, tightly regulated periplasmic protease degP. Based on the a degP-promoter and a luciferase reporter gene an online measurement technol. has been developed, allowing in vivo kinetic studies of protein misfolding during fermentation processes. The technol. was validated by periplasmic expression of a recombinant mini-antibody specific for the human EGF-receptor. Performing different feeding strategies with folding promoting agents and coexpression of the periplasmic chaperone Skp we demonstrated the amount of functional protein to be indirectly proportional to the online

luciferase signal representing the misfolded one. In this respect, the technol. offers a simple tool to evaluate and improve the yield of functionally expressed proteins in the periplasm, depending on the used folding strategy. The invention offers a technol. to improve the yield of functionally expressed recombinant proteins.

L6 ANSWER 4 OF 7 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001642903 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11676610
TITLE: Production of correctly folded Fab antibody fragment in the cytoplasm of Escherichia coli trxB gor mutants via the coexpression of molecular chaperones.
AUTHOR: Levy R; Weiss R; Chen G; Iverson B L; Georgiou G
CORPORATE SOURCE: Institute for Cell and Molecular Biology, University of Texas, Austin, 79712, USA.
CONTRACT NUMBER: R01 GM55090/04 (NIGMS)
SOURCE: Protein expression and purification, (2001 Nov) Vol. 23, No. 2, pp. 338-47.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 7 Nov 2001
Last Updated on STN: 24 Jan 2002
Entered Medline: 31 Dec 2001

AB Disulfide bonds are normally formed after a polypeptide has been exported from the reducing environment of the cytoplasm into a more oxidizing compartment, such as the bacterial periplasm. Recently, we showed that in Escherichia coli trxB gor mutants, in which the reduction of thioredoxin and glutathione is impaired, the redox potential of the cytoplasm becomes comparable to that of the mammalian endoplasmic reticulum, thus allowing the formation of disulfide bonds in certain complex proteins (P. H. Bessette et al., 1999, Proc. Natl. Acad. Sci. USA 96, 13703-13708]. Here, we investigate the expression of a Fab antibody fragment in the bacterial cytoplasm. The effect of coexpressing cytoplasmic chaperones (GroEL/ES, trigger factor, DnaK/J), as well as signal sequenceless versions of periplasmic chaperones (DsbC and Skp), was examined. Skp coexpression was shown to have the most significant effect (five- to sixfold increase) on the yield of correctly folded Fab. A maximum yield of 0.8 mg Fab/L/OD(600) Fab was obtained, indicating that cytoplasmic expression may be a viable alternative for the preparative production of antibody fragments.

L6 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000287591 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10748200
TITLE: The periplasmic Escherichia coli peptidylprolyl cis,trans-isomerase FkpA. I. Increased functional expression of antibody fragments with and without cis-prolines.
AUTHOR: Bothmann H; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
SOURCE: The Journal of biological chemistry, (2000 Jun 2) Vol. 275, No. 22, pp. 17100-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20 Jul 2000
Last Updated on STN: 20 Jul 2000
Entered Medline: 11 Jul 2000

AB The production of recombinant proteins in the periplasm of Escherichia coli can be limited by folding problems, leading to periplasmic aggregates. We used a selection system for periplasmic chaperones based on the coexpression of an E. coli library with a poorly expressing antibody single-chain Fv (scFv) fragment displayed on filamentous phage (Bothmann, H., and Pluckthun, A. (1998) Nature Biotechnol. 16, 376-380). By selection for a functional antibody, the protein Skp had been enriched previously and shown to improve periplasmic expression of a wide range of scFv fragments. This selection strategy was now repeated with a library constructed from the genomic DNA of an skp-deficient strain, leading to enrichment of the periplasmic peptidylprolyl cis,trans-isomerase (PPIase) FkpA. Coexpression of FkpA increased the amount of fusion protein displayed on the phage and dramatically improved functional periplasmic expression even of scFv fragments not containing cis-prolines. In contrast, the coexpression of the periplasmic PPIases PpiA and SurA showed no increase in the functional scFv fragment level in the periplasm or displayed on phage. Together with the in vitro data in the accompanying paper (Ramm, K., and Pluckthun, A. (2000) J. Biol. Chemical 275, 17106-17113), we conclude that the effect of FkpA is independent of its PPIase activity.

L6 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:303258 CAPLUS
DOCUMENT NUMBER: 130:307542
TITLE: A bacterial chaperonin increasing the efficiency of transport of proteins into the periplasmic space
INVENTOR(S): Pluckthun, Andreas; Bothmann, Hendrick
PATENT ASSIGNEE(S): Switz.
SOURCE: PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9922010	A1	19990506	WO 1998-EP6755		19981023
W: CA, JP, US					
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
CA 2305631	AA	19990506	CA 1998-2305631		19981023
EP 1025246	A1	20000809	EP 1998-958867		19981023
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI					
JP 2001520890	T2	20011106	JP 2000-518101		19981023
US 6630317	B1	20031007	US 2000-564351		20000501
US 2004152103	A1	20040805	US 2003-643083		20030819
PRIORITY APPLN. INFO.:			EP 1997-118457	A	19971023
			WO 1998-EP6755	W	19981023
			US 2000-564351	A3	20000501

AB A bacterial chaperonin that increases the efficiency of transport of proteins into the periplasmic space is identified

and a gene encoding it is cloned. The protein may be useful in increasing yields in the manufacture of proteins in bacterial hosts and in the development of phage display libraries. The protein appears to inhibit protein aggregation in the cytoplasm. Methods of identifying these proteins by assaying for improved folding of a reporter moiety are described. The genes may be used in combination to increase the yields of correctly folded proteins in the periplasm. Chaperonins increasing the efficiency of folding of a poorly-folding single-chain antibody were screened for using a phasmid library that carried the reporter gene. Repeated rounds of selection by panning identified the genes Skp, FkpA, and SlyX.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 7 OF 7 MEDLINE on STN
ACCESSION NUMBER: 1998216571 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9555730
TITLE: Selection for a periplasmic factor improving phage display and functional periplasmic expression.
AUTHOR: Bothmann H; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: Nature biotechnology, (1998 Apr) Vol. 16, No. 4, pp. 376-80.
Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 11 Jun 1998
Last Updated on STN: 11 Jun 1998
Entered Medline: 29 May 1998

AB The efficiency of both phage display in Escherichia coli and periplasmic expression of recombinant proteins may be limited by the same periplasmic folding steps. To search for E. coli factors that improve the efficiency of both procedures, a library of E. coli proteins was coexpressed in a phagemid vector that contained a poorly folding single-chain Fv antibody (scFv) fragment fused to g3p. We enriched, by panning for antigen binding, those phagemids in which the amount of displayed scFv is highest. We thus identified the periplasmic protein Skp/OmpH/HlpA as improving phage display of a wide range of scFv fragments. This occurs as a result of an increase in the amount of hybrid protein displayed on the phage. Coexpression of skp also increases the functional yield of scFv fragments when expressed by secretion to the periplasm.

=> l7 not l6
L8 4 L7 NOT L6

=> d ibib abs l8 1-4

L8 ANSWER 1 OF 4 MEDLINE on STN
ACCESSION NUMBER: 2006061874 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16325271
TITLE: Expression of a functional scFv fragment of an anti-idiotypic antibody with a beta-lactam hydrolytic activity.
AUTHOR: Padiolleau-Lefevre Severine; Debat Helene; Phichith Denis; Thomas Daniel; Friboulet Alain; Avalle Berangere

CORPORATE SOURCE: Genie Enzymatique et Cellulaire, UMR 6022 CNRS, Universite de Technologie de Compiegne, BP 20529, 60205 Compiegne Cedex, France.
SOURCE: Immunology letters, (2006 Feb 28) Vol. 103, No. 1, pp. 39-44. Electronic Publication: 2005-11-02.
Journal code: 7910006. ISSN: 0165-2478.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200604
ENTRY DATE: Entered STN: 1 Feb 2006
Last Updated on STN: 27 Apr 2006
Entered Medline: 26 Apr 2006

AB The single chain variable fragment (scFv) of an anti-idiotypic catalytic monoclonal antibody, 9G4H9, displaying a beta-lactamase-like activity was cloned. The recombinant protein was expressed through the periplasm in Escherichia coli in the presence or in the absence of FkpA, a chaperone-like enzyme and tested for its hydrolytic activity. The results show that the catalytic parameters for hydrolysis of ampicillin by scFv9G4H9 are clearly influenced by the presence of FkpA, indicating that the correct folding of the fragment represents a crucial step for catalysis.

L8 ANSWER 2 OF 4 MEDLINE on STN
ACCESSION NUMBER: 2004232131 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15130125
TITLE: Structural tolerance of bacterial autotransporters for folded passenger protein domains.
AUTHOR: Veiga Esteban; de Lorenzo Victor; Fernandez Luis Angel
CORPORATE SOURCE: Departmento de Biotecnologia Microbiana, Centro Nacional de Biotecnologia, Consejo Superior de Investigaciones Cientificas, Campus de Cantoblanco, Madrid 28049, Spain.
SOURCE: Molecular microbiology, (2004 May) Vol. 52, No. 4, pp. 1069-80.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200410
ENTRY DATE: Entered STN: 10 May 2004
Last Updated on STN: 29 Oct 2004
Entered Medline: 28 Oct 2004

AB In this report we investigate the capacity of bacterial autotransporters (AT) to translocate folded protein domains across the outer membrane (OM). Polypeptides belonging to the AT family contain a C-terminal domain that supports the secretion of the N-domain (the passenger) across the OM of Gram-negative bacteria. Despite some controversial data, it has been widely accepted that N-passenger domains of AT must be unfolded and devoid of disulphide bonds for efficient translocation. To address whether or not AT are able to translocate folded protein domains across the OM, we employed several types of recombinant antibodies as heterologous N-passengers of the transporter C-domain of IgA protease (C-IgAP) of Neisseria gonorroheae. The N-domains used were single chain Fv fragments (scFv) and variable mono-domains derived from camel antibodies (V(HH)) selected on the basis of their distinct and defined folding properties (i.e. enhanced solubility, stability and presence or not of disulphide bonds). Expression of these hybrids in Escherichia coli shows that stable scFv and V(HH) domains are efficiently (>99%) translocated towards the bacterial surface regardless of the presence or not of disulphide bonds on their

structure. Antigen-binding assays demonstrate that surface-exposed scFv and V(HH) domains are correctly folded and thus able to bind their cognate antigens. Expression of scFv- or V(HH)-C-IgAP hybrids in E. coli dsbA or fkpA mutant cells reveals that these periplasmic protein chaperones fold these N-domains before their translocation across the OM. Furthermore, large N-passengers composed of strings of V(HH) domains were secreted in a folded state by AT with no loss of efficacy (>99%) despite having multiple disulphide bonds. Thus AT can efficiently translocate toward the cell surface folded N-passengers composed of one, two or three immunoglobulin (Ig) domains, each with a folded diameter between approximately 2 nm and having disulphide bonds. This tolerance for folded protein domains of approximately 2 nm fits with the diameter of the central hydrophilic channel proposed for the ring-like oligomeric complex assembled by C-IgAP in the OM.

L8 ANSWER 3 OF 4 MEDLINE on STN
ACCESSION NUMBER: 2000287592 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10748201
TITLE: The periplasmic Escherichia coli peptidylprolyl cis,trans-isomerase FkpA. II. Isomerase-independent chaperone activity in vitro.
AUTHOR: Ramm K; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
SOURCE: The Journal of biological chemistry, (2000 Jun 2) Vol. 275, No. 22, pp. 17106-13.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20 Jul 2000
Last Updated on STN: 20 Jul 2000
Entered Medline: 11 Jul 2000

AB We recently identified FkpA by selecting for the increased yield of antibody single-chain Fv (scFv) fragments in phage display, even of those not containing cis-prolines. We have now investigated the properties of FkpA in vitro. The peptidylprolyl cis-trans-isomerase activity of FkpA was found to be among the highest of any such enzyme with a protein substrate, yet FkpA is not able to enhance the proline-limited refolding rate of the disulfide-free hu4D5-8 scFv fragment, probably due to inaccessibility of Pro-L95. Nevertheless, the yield of the soluble and functional scFv fragment was dramatically increased in vitro in the presence of FkpA. Similar effects were observed for an scFv fragment devoid of cis-prolines. We are thus forced to conclude that the observed folding-assisting function is independent of the isomerase activity of the protein. The beneficial effect of FkpA was found to be due to two components. First, FkpA interacts with early folding intermediates, thus preventing their aggregation. Additionally, it has the ability to reactivate inactive protein, possibly also by binding to a partially unfolded species that may exist in equilibrium with the aggregated form, which may thus be released on a productive pathway. These in vitro measurements therefore fully reflect the in vivo results from periplasmic overexpression of FkpA.

L8 ANSWER 4 OF 4 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:423660 BIOSIS

DOCUMENT NUMBER: PREV200100423660
TITLE: High enzymatic activity and chaperone function are mechanistically related features of the dimeric E. coli peptidyl-prolyl-isomerase FkpA.
AUTHOR(S): Ramm, Kathrin; Pluckthun, Andreas [Reprint author]
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstr. 190, CH-8057, Zurich, Switzerland plueckthun@biocfebs.unizh.ch
SOURCE: Journal of Molecular Biology, (6 July, 2001) Vol. 310, No. 2, pp. 485-498. print.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Sep 2001
Last Updated on STN: 22 Feb 2002

AB We have recently described the existence of a chaperone activity for the dimeric peptidyl-prolyl cis/trans isomerase FkpA from the periplasm of Escherichia coli that is independent of its isomerase activity. We have now investigated the molecular mechanism of these two activities in vitro in greater detail. The isomerase activity with a protein substrate (RNaseT1) is characterized by a 100-fold higher kcat/KM value than with a short tetrapeptide substrate. This enhanced activity with a protein is due to an increased affinity towards the protein substrate mediated by a polypeptide-binding site that is distinct from the active site. The chaperone activity is also mediated by interaction of folding and unfolding intermediates with a binding site that is most likely identical to the polypeptide-binding site which enhances catalysis. Both activities are thus mechanistically related, being based on the transient interaction with this high-affinity polypeptide-binding site. Only the isomerase activity, but not the chaperone activity, with the substrate citrate synthase can be inhibited by FK520. Experiments with the isolated domains of FkpA imply that both the isomerase and the chaperone site are located on the highly conserved FKBP domain. The additional amino-terminal domain mediates the dimerization and thus places the two active sites of the FKBP domains in juxtaposition, such that they can simultaneously interact with a protein, and this is required for full catalytic activity.

=> l5 and peptidyl and prolyl
L9 1 L5 AND PEPTIDYL AND PROLYL

=> d ibib abs l9

L9 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:423660 BIOSIS
DOCUMENT NUMBER: PREV200100423660
TITLE: High enzymatic activity and chaperone function are mechanistically related features of the dimeric E. coli peptidyl-prolyl-isomerase FkpA.
AUTHOR(S): Ramm, Kathrin; Pluckthun, Andreas [Reprint author]
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstr. 190, CH-8057, Zurich, Switzerland plueckthun@biocfebs.unizh.ch
SOURCE: Journal of Molecular Biology, (6 July, 2001) Vol. 310, No. 2, pp. 485-498. print.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Sep 2001
Last Updated on STN: 22 Feb 2002

AB We have recently described the existence of a chaperone activity for the dimeric peptidyl-prolyl cis/trans isomerase FkpA from the periplasm of Escherichia coli that is independent of its isomerase activity. We have now investigated the molecular mechanism of these two activities in vitro in greater detail. The isomerase activity with a protein substrate (RNaseT1) is characterized by a 100-fold higher kcat/KM value than with a short tetrapeptide substrate. This enhanced activity with a protein is due to an increased affinity towards the protein substrate mediated by a polypeptide-binding site that is distinct from the active site. The chaperone activity is also mediated by interaction of folding and unfolding intermediates with a binding site that is most likely identical to the polypeptide-binding site which enhances catalysis. Both activities are thus mechanistically related, being based on the transient interaction with this high-affinity polypeptide-binding site. Only the isomerase activity, but not the chaperone activity, with the substrate citrate synthase can be inhibited by FK520. Experiments with the isolated domains of FkpA imply that both the isomerase and the chaperone site are located on the highly conserved FKBP domain. The additional amino-terminal domain mediates the dimerization and thus places the two active sites of the FKBP domains in juxtaposition, such that they can simultaneously interact with a protein, and this is required for full catalytic activity.

=> d his

(FILE 'HOME' ENTERED AT 19:34:20 ON 19 JUL 2006)

FILE 'MEDLINE, BIOSIS, CAPLUS, SCISEARCH, EMBASE, WPIDS' ENTERED AT 19:34:47 ON 19 JUL 2006

L1 95 PERIPLASM? AND CHAPERON? AND FOLD? AND (HETEROLOGOUS OR ANTIBOD
L2 18 SKP AND L1
L3 22 FKP? AND L1
L4 12 L2 NOT L3
L5 34 DUP REM L1 (61 DUPLICATES REMOVED)
L6 7 DUP REM L2 (11 DUPLICATES REMOVED)
L7 6 DUP REM L3 (16 DUPLICATES REMOVED)
L8 4 L7 NOT L6
L9 1 L5 AND PEPTIDYL AND PROLYL

=> l5 not l6
L10 27 L5 NOT L6

=> l10 not l7
L11 23 L10 NOT L7

=> l11 not l9
L12 23 L11 NOT L9

=> d ibib abs l12 1-23

L12 ANSWER 1 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2006183933 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 16253369
TITLE: Coexpression of TorD enhances the transport of GFP via the TAT pathway.
AUTHOR: Li Si-Yu; Chang Bang-Yang; Lin Sung-Chyr
CORPORATE SOURCE: Department of Chemical Engineering, National Chung Hsing University, Taichung 402, Taiwan.
SOURCE: Journal of biotechnology, (2006 Apr 20) Vol. 122, No. 4, pp. 412-21. Electronic Publication: 2005-10-25.

Journal code: 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 4 Apr 2006
Last Updated on STN: 4 May 2006

AB Twin-arginine translocation (Tat) pathway is capable of secreting fully folded proteins into the periplasm of Gram-negative bacteria and may thus be an ideal system for the expression of active cofactor-containing proteins. However, the applications of Tat system for such purpose have been plagued by low translocation efficiencies. In this study, we demonstrate that the coexpression of a soluble chaperone , TorD, in conjunction with the TorA signal peptide, the translocation efficiency of GFP can be enhanced by more than three-fold. The enhancement in translocation efficiency is believed to be a result of reduced proteolysis mediated by the binding of TorD toward the TorA signal peptide. We believe this approach can be further exploited for the expression and secretion of other heterologous proteins as well as traditional Tat substrate proteins.

L12 ANSWER 2 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2005523855 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16095898
TITLE: Preparative expression of secreted proteins in bacteria: status report and future prospects.
AUTHOR: Georgiou George; Segatori Laura
CORPORATE SOURCE: Department of Chemical Engineering, University of Texas, Austin, USA.. gg@che.utexas.edu
CONTRACT NUMBER: GM-55090-05 (NIGMS)
SOURCE: Current opinion in biotechnology, (2005 Oct) Vol. 16, No. 5, pp. 538-45. Ref: 57
Journal code: 9100492. ISSN: 0958-1669.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200512
ENTRY DATE: Entered STN: 4 Oct 2005
Last Updated on STN: 20 Dec 2005
Entered Medline: 16 Dec 2005

AB The expression of heterologous secreted proteins in Escherichia coli is widely employed for laboratory and preparative purposes. Thanks to advances in expression technologies over the past 25 years, many mammalian proteins can now be produced routinely in secreted form with yields in the gram/litre scale. Nonetheless, ensuring efficient secretion across the inner membrane, and preventing proteolytic degradation, incorrect disulfide-bond formation and aggregation into periplasmic inclusion bodies, frequently presents significant challenges. Recent advances in the understanding of the periplasmic folding quality control system are leading to new strategies to facilitate the expression of heterologous secreted proteins. In parallel, protein design and directed evolution approaches are beginning to be exploited for engineering of the cellular protein folding machinery to achieve further improvements in protein expression.

L12 ANSWER 3 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2001482671 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11525996
TITLE: Cosecretion of chaperones and low-molecular-size

medium additives increases the yield of recombinant disulfide-bridged proteins.
AUTHOR: Schaffner J; Winter J; Rudolph R; Schwarz E
CORPORATE SOURCE: Institut fur Biotechnologie, Martin-Luther-Universitat Halle-Wittenberg, 06120 Halle, Germany.
SOURCE: Applied and environmental microbiology, (2001 Sep) Vol. 67, No. 9, pp. 3994-4000.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 30 Aug 2001
Last Updated on STN: 22 Jan 2002
Entered Medline: 4 Dec 2001

AB Attempts were made to engineer the periplasm of Escherichia coli to an expression compartment of heterologous proteins in their native conformation. As a first approach the low-molecular-size additive L-arginine and the redox compound glutathione (GSH) were added to the culture medium. Addition of 0.4 M L-arginine and 5 mM reduced GSH increased the yield of a native tissue-type plasminogen activator variant (rPA), consisting of the kringle-2 and the protease domain, and a single-chain antibody fragment (scFv) up to 10- and 37-fold, respectively. A variety of other medium additives also had positive effects on the yield of rPA. In a second set of experiments, the effects of cosecreted ATP-independent molecular chaperones on the yields of native therapeutic proteins were investigated. At optimized conditions, cosecretion of E. coli DnaJ or murine Hsp25 increased the yield of native rPA by a factor of 170 and 125, respectively. Cosecretion of DnaJ also dramatically increased the amount of a second model protein, native proinsulin, in the periplasm. The results of this study are anticipated to initiate a series of new approaches to increase the yields of native, disulfide-bridged, recombinant proteins in the periplasm of E. coli.

L12 ANSWER 4 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2001472732 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11282637
TITLE: Secretion of recombinant proteins via the chaperone /usher pathway in Escherichia coli.
AUTHOR: Zavialov A V; Batchikova N V; Korpela T; Petrovskaya L E; Korobko V G; Kersley J; MacIntyre S; Zav'yalov V P
CORPORATE SOURCE: Finnish-Russian Joint Biotechnology Laboratory, University of Turku, BioCity 6A, FIN-20520 Turku, Finland.. azaviabo@abo.fi
SOURCE: Applied and environmental microbiology, (2001 Apr) Vol. 67, No. 4, pp. 1805-14.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 27 Aug 2001
Last Updated on STN: 27 Aug 2001
Entered Medline: 23 Aug 2001

AB F1 antigen (Caf1) of Yersinia pestis is assembled via the Caf1M chaperone/Caf1A usher pathway. We investigated the ability of this assembly system to facilitate secretion of full-length heterologous proteins fused to the Caf1 subunit in Escherichia coli. Despite correct processing of a chimeric protein composed of a

modified Caf1 signal peptide, mature human interleukin-1beta (hIL-1beta), and mature Caf1, the processed product (hIL-1beta:Caf1) remained insoluble. Coexpression of this chimera with a functional Caf1M chaperone led to the accumulation of soluble hIL-1beta:Caf1 in the periplasm. Soluble hIL-1beta:Caf1 reacted with monoclonal antibodies directed against structural epitopes of hIL-1beta. The results indicate that Caf1M-induced release of hIL-1beta:Caf1 from the inner membrane promotes folding of the hIL-1beta domain. Similar results were obtained with the fusion of Caf1 to hIL-1beta receptor antagonist or to human granulocyte-macrophage colony-stimulating factor. Following coexpression of the hIL-1beta:Caf1 precursor with both the Caf1M chaperone and Caf1A outer membrane protein, hIL-1beta:Caf1 could be detected on the cell surface of E. coli. These results demonstrate for the first time the potential application of the chaperone/usher secretion pathway in the transport of subunits with large heterogeneous N-terminal fusions. This represents a novel means for the delivery of correctly folded heterologous proteins to the periplasm and cell surface as either polymers or cleavable monomeric domains.

L12 ANSWER 5 OF 23 MEDLINE on STN
ACCESSION NUMBER: 2001080102 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11099799
TITLE: Bacterial lipases from Pseudomonas: regulation of gene expression and mechanisms of secretion.
AUTHOR: Rosenau F; Jaeger K
CORPORATE SOURCE: Ruhr-Universitat Bochum, Lehrstuhl Biologie der Mikroorganismen, Universitatsstrasse 150, 44780, Bochum, Germany.
SOURCE: Biochimie, (2000 Nov) Vol. 82, No. 11, pp. 1023-32. Ref: 80
Journal code: 1264604. ISSN: 0300-9084.
PUB. COUNTRY: France
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 11 Jan 2001

AB Lipases from Pseudomonas bacteria are widely used for a variety of biotechnological applications. Overexpression in heterologous hosts like Escherichia coli failed to produce enzymatically active lipase prompting to study the molecular mechanisms underlying the regulation of lipase gene expression and secretion. The prototype lipase from P. aeruginosa is encoded in a bicistronic operon which is transcribed from two different promotors, one of which depends on the alternative sigma factor RpoN (sigma(54)). Recently, a two-component regulatory system was identified as an element controlling transcription of the lipase operon. P. aeruginosa lipase is secreted via a type II pathway. The cytoplasmic prelipase contains a 26 amino acid N-terminal signal sequence mediating secretion across the inner membrane via the Sec-machinery. In the periplasm, lipase folds into an enzymatically active conformation assisted by its specific intermolecular chaperone Lif and by unspecific accessory folding catalysts including Dsb-proteins which catalyze the formation of a disulfide bond. Enzymatically active and secretion-competent lipase is finally transported through a complex secretion machinery consisting of 12 different Xcp-proteins of which XcpQ forms a pore-like structure in the outer membrane allowing the release of lipase into the extracellular medium. Biotechnologically important lipases from Burkholderia glumae and P.

alcaligenes also use such a type II secretion pathway whereas lipases from P. fluorescens and Serratia marcescens, which lack a typical signal sequence are secreted via a type I pathway. Future challenges to produce Pseudomonas lipases may include artificial up-regulation of lipase gene transcription and construction of more efficient expression strains in which both folding and secretion of lipase are optimized.

L12 ANSWER 6 OF 23 MEDLINE on STN
ACCESSION NUMBER: 1999380793 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10449317
TITLE: Improving protein secretion by engineering components of the bacterial translocation machinery.
AUTHOR: Braun P; Gerritse G; van Dijl J M; Quax W J
CORPORATE SOURCE: Pharmaceutical Biology, University Centre for Pharmacy, Antonius Deusinglaan 1, 9713, AV Groningen, The Netherlands.
SOURCE: Current opinion in biotechnology, (1999 Aug) Vol. 10, No. 4, pp. 376-81. Ref: 35
Journal code: 9100492. ISSN: 0958-1669.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 25 Sep 1999
Last Updated on STN: 25 Sep 1999
Entered Medline: 13 Sep 1999

AB The increased insight into the mechanism of bacterial protein translocation has resulted in new concepts for the production of heterologous proteins. The periplasm of gram-negative bacteria is revealed to have a role as a 'protein construction compartment', which can be used to fold complex proteins. Passage across the outer membrane, however, remains a challenge due to the high selectivity of the outer membrane translocase. In gram-positive bacteria, slow folding at the membrane-cell-wall interface can make heterologous proteins vulnerable to degradation by wall-associated proteases. The recent identification of thiol-disulfide oxidoreductases in Bacillus subtilis might open the possibility of secreting proteins containing multiple disulfide bonds from this host.

L12 ANSWER 7 OF 23 MEDLINE on STN
ACCESSION NUMBER: 1999201131 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10099335
TITLE: Generating controlled reducing environments in aerobic recombinant Escherichia coli fermentations: effects on cell growth, oxygen uptake, heat shock protein expression, and in vivo CAT activity.
AUTHOR: Gill R T; Cha H J; Jain A; Rao G; Bentley W E
CORPORATE SOURCE: Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute and Department of Chemical Engineering, University of Maryland, College Park, Maryland 20742, USA.
SOURCE: Biotechnology and bioengineering, (1998 Jul 20) Vol. 59, No. 2, pp. 248-59.
Journal code: 7502021. ISSN: 0006-3592.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 17 May 1999

Last Updated on STN: 17 May 1999
Entered Medline: 4 May 1999

AB The independent control of culture redox potential (CRP) by the regulated addition of a reducing agent, dithiothreitol (DTT) was demonstrated in aerated recombinant Escherichia coli fermentations. Moderate levels of DTT addition resulted in minimal changes to specific oxygen uptake, growth rate, and dissolved oxygen. Excessive levels of DTT addition were toxic to the cells resulting in cessation of growth. Chloramphenicol acetyltransferase (CAT) activity (nmoles/microgram total protein min.) decreased in batch fermentation experiments with respect to increasing levels of DTT addition. To further investigate the mechanisms affecting CAT activity, experiments were performed to assay heat shock protein expression and specific CAT activity (nmoles/microgram CAT min.). Expression of such molecular chaperones as GroEL and DnaK were found to increase after addition of DTT. Additionally, sigma factor 32 (sigma32) and several proteases were seen to increase dramatically during addition of DTT. Specific CAT activity (nmoles/microgram CAT min.) varied greatly as DTT was added, however, a minimum in activity was found at the highest level of DTT addition in E. coli strains RR1 [pBR329] and JM105 [pROEX-CAT]. In conjunction, cellular stress was found to reach a maximum at the same levels of DTT. Although DTT addition has the potential for directly affecting intracellular protein folding, the effects felt from the increased stress within the cell are likely the dominant effector. That the effects of DTT were measured within the cytoplasm of the cell suggests that the periplasmic redox potential was also altered. The changes in specific CAT activity, molecular chaperones, and other heat shock proteins, in the presence of minimal growth rate and oxygen uptake alterations, suggest that the ex vivo control of redox potential provides a new process for affecting the yield and conformation of heterologous proteins in aerated E. coli fermentations.

L12 ANSWER 8 OF 23 MEDLINE on STN

ACCESSION NUMBER: 1998268994 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9603868
TITLE: Enhanced secretory production of a single-chain antibody fragment from Bacillus subtilis by coproduction of molecular chaperones.
AUTHOR: Wu S C; Ye R; Wu X C; Ng S C; Wong S L
CORPORATE SOURCE: Department of Biological Sciences, Division of Cellular, Molecular and Microbial Biology, University of Calgary, Calgary, Alberta T2N 1N4, Canada.
SOURCE: Journal of bacteriology, (1998 Jun) Vol. 180, No. 11, pp. 2830-5.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 8 Jul 1998
Last Updated on STN: 8 Jul 1998
Entered Medline: 25 Jun 1998

AB Formation of inclusion bodies is a major limiting factor for secretory production of an antidigoxin single-chain antibody (SCA) fragment from Bacillus subtilis. To address this problem, three new strains with enhanced production of molecular chaperones were constructed. WB600BHM constitutively produces the major intracellular molecular chaperones in an appropriate ratio without any heat shock treatment. This strain reduced the formation of insoluble SCA by 45% and increased the secretory production yield by 60%. The second

strain, WB600B[pEPP], overproduces an extracytoplasmic molecular chaperone, PrsA. An increase in the total yield of SCA was observed. The third strain, WB600BHM[pEPP], coproduces both intracellular and extracytoplasmic molecular chaperones. This led to a further reduction in inclusion body formation and a 2.5-fold increase in the secretory production yield. SCA fragments secreted by this strain were biologically active and showed affinity to digoxin comparable to the affinity of those secreted by strains without overproduction of molecular chaperones. Interestingly, accumulation of a pool of periplasmic SCA was observed in the PrsA-overproducing strains. This pool is suggested to represent the secreted folding intermediates in the process of achieving their final configuration.

L12 ANSWER 9 OF 23 MEDLINE on STN
ACCESSION NUMBER: 96209987 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8631865
TITLE: Eukaryotic protein disulfide isomerase complements Escherichia coli dsbA mutants and increases the yield of a heterologous secreted protein with disulfide bonds.
AUTHOR: Ostermeier M; De Sutter K; Georgiou G
CORPORATE SOURCE: Department of Chemical Engineering, University of Texas, Austin 78712, USA.
CONTRACT NUMBER: GM47520-01 (NIGMS)
SOURCE: The Journal of biological chemistry, (1996 May 3) Vol. 271, No. 18, pp. 10616-22.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 15 Jul 1996
Last Updated on STN: 29 Jan 1999
Entered Medline: 1 Jul 1996

AB Eukaryotic protein disulfide isomerase (PDI) is a 55-kDa enzyme with cysteine oxidoreductase, chaperone, and antichaperone activities that catalyzes disulfide formation and rearrangement in the eukaryotic endoplasmic reticulum. In Gram-negative bacteria, the formation of disulfide bonds in the periplasm is mediated by DsbA, a strong cysteine oxidase but an inefficient catalyst of disulfide bond isomerization with no known chaperone activity. We show that rat PDI (rPDI) secreted in the periplasmic space of Escherichia coli can catalyze the formation of disulfide bonds and complement several of the phenotypes of dsbA mutants. The function of rPDI was dependent on the dsbB gene, suggesting that the reoxidation of this eukaryotic enzyme involves direct interactions with bacterial redox proteins. Co-expression of rPDI increased the yield of bovine pancreatic trypsin inhibitor (BPTI) severalfold, an effect that was enhanced when reduced glutathione was added to the growth medium. Whereas PDI is thought to function primarily as an isomerase in the eukaryotic endoplasmic reticulum, rPDI failed to decrease the accumulation of two-disulfide folding intermediates of BPTI and thus did not appear to appreciably catalyze the rate-limiting step in the oxidative folding pathway of BPTI. These results demonstrate that expression of eukaryotic foldases in E. coli can be exploited to better understand their function in vivo and also to increase the yield of biotechnologically valuable proteins.

L12 ANSWER 10 OF 23 MEDLINE on STN
ACCESSION NUMBER: 95018232 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7932722
TITLE: Correctly folded T-cell receptor fragments in the

periplasm of Escherichia coli. Influence of folding catalysts.
AUTHOR: Wulfing C; Pluckthun A
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Protein Engineering Group, Martinsried, Germany.
SOURCE: Journal of molecular biology, (1994 Oct 7) Vol. 242, No. 5, pp. 655-69.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 22 Dec 1994
Last Updated on STN: 22 Dec 1994
Entered Medline: 27 Oct 1994

AB The T-cell receptor is the central recognition molecule in cellular immunity. Its extracellular domains are homologous with and thought to be structurally similar to an antibody Fab fragment. Despite the biological importance of the TCR and the ease of bacterial expression of antibody fragments, there are only few reports of TCR-fragment expression in E. coli. In order to understand the difficulties of expressing correctly folded TCR fragments in E. coli, we have characterized the expression behavior of single-chain Fv analogs of three different TCRs (scTCR). All of them can be folded into the correct conformation in the periplasm of E. coli, yet the extent of correct folding varies greatly. In order to overcome the folding problems of some of the scTCRs, we have developed a system with enhanced in vivo folding capability based on the simultaneous induction of the heat-shock response and over-expression of the E. coli disulfide isomerase DsbA at low temperature. We present a model describing the folding of the scTCRs in the periplasm of E. coli and possible points of folding assistance. The role of the periplasm as an independent folding compartment is emphasized and the existence of a general periplasmic chaperone is postulated. We have also shown that a bivalent scTCR, dimerized in vivo with helix-turn-helix modules, can be expressed in a correctly folded form.

L12 ANSWER 11 OF 23 MEDLINE on STN
ACCESSION NUMBER: 94328921 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8052121
TITLE: Protein folding in the periplasm of Escherichia coli.
AUTHOR: Wulfing C; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: Molecular microbiology, (1994 Jun) Vol. 12, No. 5, pp. 685-92. Ref: 96
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 14 Sep 1994
Last Updated on STN: 14 Sep 1994
Entered Medline: 6 Sep 1994

AB With the discovery of molecular chaperones and the development of heterologous gene expression techniques, protein folding in bacteria has come into focus as a potentially limiting factor in expression and as a topic of interest in its own right. Many

proteins of importance in biotechnology contain disulphide bonds, which form in the Escherichia coli periplasm, but most work on protein folding in the periplasm of E. coli is very recent and is often speculative. This MicroReview gives a short overview of the possible fates of a periplasmic protein from the moment it is translocated, as well as of the E. coli proteins involved in this process. After an introduction to the specific physiological situation in the periplasm of E. coli, we discuss the proteins that might help other proteins to obtain their correctly folded conformation--disulphide isomerase, rotamase, parts of the translocation apparatus and putative periplasmic chaperones--and briefly cover the guided assembly of multi-subunit structures. Finally, our MicroReview turns to the fate of misfolded proteins: degradation by periplasmic proteases and aggregation phenomena.

L12 ANSWER 12 OF 23 MEDLINE on STN
ACCESSION NUMBER: 94242403 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7910466
TITLE: Intra- and extracellular expression of an scFv antibody fragment in E. coli: effect of bacterial strains and pathway engineering using GroES/L chaperonins.
AUTHOR: Duenas M; Vazquez J; Ayala M; Soderlind E; Ohlin M; Perez L; Borrebaeck C A; Gavilondo J V
CORPORATE SOURCE: Center for Genetic Engineering and Biotechnology, Havana, Cuba.
SOURCE: BioTechniques, (1994 Mar) Vol. 16, No. 3, pp. 476-7, 480-3. Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 29 Jun 1994
Last Updated on STN: 6 Feb 1995
Entered Medline: 22 Jun 1994

AB We have studied the influence of bacterial host on the secretion of single-chain Fv antibody fragment (scFv), the production of this antibody fragment as intracellular fusion protein, and the effect of chaperonin coexpression on intracellular antibody expression. Seven bacterial strains were transformed with a vector carrying the genes encoding the variable regions of an anti-CEA scFv antibody and the ompA leader sequence (ptrp/ompA/scFvCEA). Expression and secretion of this antibody fragment were highest in the W3110 strain, as determined by Western blot analysis and enzyme immunoassay, where the scFv fragment amounted to approximately 30% of the total periplasmic protein. Except for BMH71-18, the other strains were unsuitable for antibody fragment expression, suggesting screening of bacterial strains as an important parameter. For intracellular expression, the scFv was expressed as a fusion protein with a 26-amino acid N-terminal fragment of human interleukin-2 (IL-2), using the pIL-2f/scFvCEA vector. The fusion protein was expressed at 30% of total biomass and retained antigen binding after in vitro refolding. Co-expression of chaperonin-encoding plasmid pGroES/L with pIL-2f/scFv increased the intracellular production of the fusion protein twofold, with a similar increase in the final amount of active scFv antibody fragment that could be obtained after in vitro refolding. The chaperonins had no effect on secretion of scFv antibody fragments, using the ptrp/ompA/scFvCEA.

L12 ANSWER 13 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:295827 BIOSIS
DOCUMENT NUMBER: PREV200600294492
TITLE: Thioredoxin fusions increase folding of single chain Fv antibodies in the cytoplasm of Escherichia coli: Evidence that chaperone activity is the prime effect of thioredoxin.
AUTHOR(S): Jurado, Paola; de Lorenzo, Victor; Fernandez, Luis A. [Reprint Author]
CORPORATE SOURCE: CSIC, Ctr Nacl Biotecnol, Campus Cantoblanco, E-28049 Madrid, Spain
lafdez@cnb.uam.es
SOURCE: Journal of Molecular Biology, (MAR 17 2006) Vol. 357, No. 1, pp. 49-61.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 May 2006
Last Updated on STN: 31 May 2006

AB In this study we investigate the effect of thioredoxin (Trx1) protein fusions in the production, oxidation, and folding of single chain Fv (scFv) antibodies in the cytoplasm of Escherichia coli. We analyze the expression levels, solubility, disulfide-bond formation, and antigen-binding properties of Trx1-scFv fusions in E. coli wild-type cells and isogenic strains carrying mutations in the glutathione oxidoreductase (gor) and/or thioredoxin reductase (trxB) genes. We compare the Trx1-scFv fusions with other reported systems for production of scFv in the cytoplasm of E. coli, including protein fusions to the maltose-binding protein. In addition, we analyze the effect of co-expressing a signal-sequence-less derivative of the periplasmic chaperone and disulfide-bond isomerase DsbC (Delta ssDsbC), which has been shown to act as a chaperone for scFvs in the cytoplasm. The results reported here demonstrate that Trx1 fusions produce the highest expression level and induce the correct folding of scFvs even in the absence of Delta ssDsbC in the cytoplasm of E. coli trxB gor cells. The disulfide bridges of Trx1-scFv fusions were formed correctly in E. coli trxB gor cells, but not in trxB single mutants. Antigen-binding assays showed that Trx1 has only a minor influence in the affinity of the scFv, indicating that Trx1-scFv fusions can be used without removal of the Trx1 moiety In addition, we proved that a Trx1"AGPA" variant, having its catalytic cysteine residues mutated to alanine, was fully capable of assisting the folding of the fused scFvs. Taken together, our data indicate that the Trx1 moiety acts largely as an intramolecular protein chaperone, not as a disulfide bond catalyst, inducing the correct folding of scFvs in the cytoplasm of E. coli trxB gor cells. (c) 2005 Elsevier Ltd. All rights reserved.

L12 ANSWER 14 OF 23 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:24976 BIOSIS
DOCUMENT NUMBER: PREV200200024976
TITLE: Understanding the art of producing protein and nonprotein molecules in Escherichia coli.
AUTHOR(S): Balbas, Paulina [Reprint author]
CORPORATE SOURCE: Centro de Investigacion en Biotecnologia, Universidad Autonoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, MOR, Mexico
paulina@cib.uaem.mx
SOURCE: Molecular Biotechnology, (November, 2001) Vol. 19, No. 3, pp. 251-267. print.
ISSN: 1073-6085.
DOCUMENT TYPE: Article

General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Dec 2001
Last Updated on STN: 25 Feb 2002

AB The high-level production of functional proteins in E. coli is a very extense field of research in biotechnology. A number of important aspects to be considered in the initial design of an expression system and their interplay, were clear years ago. However, in recent times, strategies that go beyond transcription, translation, stability, vector, and strain choice, have been developed; so now expression of active peptides can be viewed as a more integrated process. Coexpression of protein subunits, foldases and chaperones, protein folding, location and purification schemes, metabolic engineering of the cell's central metabolism, and in vitro refolding strategies, are some of the novelties that are now available to aid in the success of an efficient expression system for active heterologous proteins. This review presents a compilation of the basic issues that influence the success in the production of protein and nonprotein products in Escherichia coli, as well as some general strategies designed to facilitate downstream process and improve biosynthesis yields.

L12 ANSWER 15 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:459675 CAPLUS
DOCUMENT NUMBER: 137:29970
TITLE: New procedure for the extraction of native therapeutic proteins in E. coli
AUTHOR(S): Schwarz, Elisabeth
CORPORATE SOURCE: Institut fur Biotechnologie, Martin-Luther-Universitat Halle-Wittenberg, Germany
SOURCE: BIOspektrum (2002), 8(2), 171-173
CODEN: BOSPFD; ISSN: 0947-0867
PUBLISHER: Spektrum Akademischer Verlag
DOCUMENT TYPE: Journal; General Review
LANGUAGE: German

AB A review. To investigate the effect of chaperone proteins during the in vivo structure formation of released proteins from Escherichia coli, 2 ATP-independent representatives were selected: DnaJ, a co-chaperone of DnaK and Hsp25, a heat-shock protein from mice. The encoding sequences of the chaperone proteins were fused at the N-terminus with signal sequences for the secretion into the periplasma. As a model protein, a variant of the tissue-type plasminogen activator (rPA) was chosen with 9 disulfide-bonds. A 2-3-fold increase of the yield was noticed during the co-secretion of rPA with DnaJ or Hsp25. A yield-increasing effect was also found during the co-secretion of DnaJ with pro-insulin as a model protein. In the case of a single chain-Fv-antibody fragment (scFv), L-Arg in the culture medium increased the yield of native protein. The technol. problems are discussed which arise in recombinant techniques for the production of functioning therapeutic proteins with disulfide bonds.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 16 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:179255 CAPLUS
DOCUMENT NUMBER: 136:198948
TITLE: Development of heterologous protein expression system using molecular chaperones and foldases
AUTHOR(S): Kurokawa, Yoichi; Oda, Junichi
CORPORATE SOURCE: Dept. Biosci., Fukui Pref. Univ., Yoshida-gun, Fukui-ken, 910-1195, Japan
SOURCE: Baiosaiensu to Indasutori (2002), 60(2), 99-102

CODEN: BIDSE6; ISSN: 0914-8981
PUBLISHER: Baioindasutori Kyokai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review on the solubilization of recombinant proteins by overexpression of mol. chaperones, roles of Dsb proteins in the formation of protein disulfide bonds in Escherichia coli, enhancement of periplasmic production of heterologous proteins in E. coli overexpressing Dsb proteins, and novel techniques for the production of active forms of recombinant proteins.

L12 ANSWER 17 OF 23 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2002:938230 SCISEARCH
THE GENUINE ARTICLE: 615AW
TITLE: Overexpression of DsbC and DsbG markedly improves soluble and functional expression of single-chain Fv antibodies in Escherichia coli
AUTHOR: Zhang Z; Li Z H; Wang F; Fang M; Yin C C; Zhou Z Y; Lin Q; Huang H L (Reprint)
CORPORATE SOURCE: Acad Sinica, Grp 102, Inst Genet & Dev Biol, Beijing 100101, Peoples R China (Reprint)
COUNTRY OF AUTHOR: Peoples R China
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (NOV 2002) Vol. 26, No. 2, pp. 218-228.
ISSN: 1046-5928.
PUBLISHER: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 31
ENTRY DATE: Entered STN: 6 Dec 2002
Last Updated on STN: 6 Dec 2002
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Single-chain Fv antibodies (scFv), a group of reconstructed molecules with several disulfide bonds, are prone to aggregate as inclusion bodies, the insoluble species of natural proteins, when expressed in Escherichia coli, especially at high level. Recovery of functionally active products from inclusion bodies is onerous and ineffective. We have increased the soluble and functional scFv yields by fusing either DsbC or DsbG, two E coli disulfide isomerases with general chaperone function, to scFvs. Compared to the totally insoluble inclusion bodies of scFvs expressed separately, more than half of each fusion protein DsbC-scFv or DsbG-scFv was soluble, according to SDS PAGE analysis. The more effective solubility was obtained when the fused protein DsbG-scFv was co-expressed simultaneously with DsbC under the same promoter. Under this condition, the soluble. portion of DsbG-scFv increased from about 50% to 90% measured by scanning SDS-PAGE gel. Co-expression of DsbC can change fusion protein CBD-scFv from totally insoluble when expressed in E. coli separately to a considerable portion of soluble CBD-scFv. Antigen-binding activity assay showed that scFvs retained full affinity to specific antigens. We also determined that general molecular chaperones GroEL and GroES had no effects on the solubility of scFvs when co-expressed with scFv in E. coli. We propose that the correct formation of disulfide bonds in scFvs is the crucial factor responsible for solubility of scFvs.

L12 ANSWER 18 OF 23 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2004484767 EMBASE
TITLE: Recombinant protein folding and misfolding in Escherichia coli.

AUTHOR: Baneyx F.; Mujacic M.
CORPORATE SOURCE: F. Baneyx, Depts. of Chem. Eng. and Bioeng., University of Washington, Box 351750, Seattle, WA 98195, United States. baneyx@u.washington.edu
SOURCE: Nature Biotechnology, (2004) Vol. 22, No. 11, pp. 1399-1407. .
Refs: 90
ISSN: 1087-0156 CODEN: NABIF
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
022 Human Genetics
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 2 Dec 2004
Last Updated on STN: 2 Dec 2004

AB The past 20 years have seen enormous progress in the understanding of the mechanisms used by the enteric bacterium Escherichia coli to promote protein folding, support protein translocation and handle protein misfolding. Insights from these studies have been exploited to tackle the problems of inclusion body formation, proteolytic degradation and disulfide bond generation that have long impeded the production of complex heterologous proteins in a properly folded and biologically active form. The application of this information to industrial processes, together with emerging strategies for creating designer folding modulators and performing glycosylation all but guarantee that E. coli will remain an important host for the production of both commodity and high value added proteins.

L12 ANSWER 19 OF 23 EMBASE
ACCESSION NUMBER: 96203207 EMBASE
DOCUMENT NUMBER: 1996203207
TITLE: A novel DnaJ-like protein in Escherichia coli inserts into the cytoplasmic membrane with a Type III topology.
AUTHOR: Clarke D.J.; Jacq A.; Holland I.B.
CORPORATE SOURCE: URA 1354, Institut de Genetique Microbiologie, Universite Paris-Sud,91405 Orsay Cedex, France
SOURCE: Molecular Microbiology, (1996) Vol. 20, No. 6, pp. 1273-1286. .
ISSN: 0950-382X CODEN: MOMIEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 9 Aug 1996
Last Updated on STN: 9 Aug 1996

AB We describe a novel Escherichia coli protein, DjIA, containing a highly conserved J-region motif, which is present in the DnaJ protein chaperone family and required for interaction with DnaK. Remarkably, DjIA is shown to be a membrane protein, localized to the inner membrane with the unusual Type III topology (N-out, C-in). Thus, DjIA appears to present an extremely short N-terminus to the periplasm and has a single transmembrane domain (TMD) and a large cytoplasmic domain containing the C-terminal J-region. Analysis of the TMD of DjIA and recently identified homologues in Coxiella burnetti and Haemophilus influenzae revealed a striking pattern of conserved glycines (or rarely alenine), with a four-residue spacing. This motif, predicted to form a spiral groove in the TMD, is more marked than a repeating glycine motif, implicated in the dimerization of TMDs of some eukaryotic proteins. This

feature of DjIA could represent a promiscuous docking mechanism for interaction with a variety of membrane proteins. DjIA null mutants can be isolated but these appear rapidly to accumulate suppressors to correct envelope and growth defects. Moderate (10-fold) overproduction of DjIA suppresses a mutation in FtsZ but markedly perturbs cell division and cell- envelope growth in minimal medium. We propose that DjIA plays a role in the correct assembly, activity and/or maintenance of a number of membrane proteins, including two-component signal-transduction systems.

L12 ANSWER 20 OF 23 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2001-246712 [26] WPIDS
CROSS REFERENCE: 2001-204356 [21]
DOC. NO. CPI: C2001-074349
TITLE: Producing naturally folded eukaryotic proteins e.g. antibodies, interferon, hormones or proteases that contain two or several cysteines linked by disulfide bridges comprises co-expression of a molecular chaperone.
DERWENT CLASS: B04 D16
INVENTOR(S): AMBROSIUS, D; RUDOLPH, R; SCHAFFNER, J; SCHWARZ, E; SCHAEFFNER, J
PATENT ASSIGNEE(S): (HOFF) HOFFMANN LA ROCHE & CO AG F
COUNTRY COUNT: 26
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1077262	A1	20010221	(200126)*	EN	35
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
CA 2313248	A1	20010129	(200126)	EN	
EP 1077262	B1	20050720	(200547)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
DE 60021318	E	20050825	(200557)		
ES 2247990	T3	20060316	(200622)		
DE 60021318	T2	20060524	(200635)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1077262	A1	EP 2000-115839	20000724
CA 2313248	A1	CA 2000-2313248	20000726
EP 1077262	B1	EP 2000-115839	20000724
DE 60021318	E	DE 2000-00021318	20000724
		EP 2000-115839	20000724
ES 2247990	T3	EP 2000-115839	20000724
DE 60021318	T2	DE 2000-00021318	20000724
		EP 2000-115839	20000724

FILING DETAILS:

PATENT NO	KIND		PATENT NO
DE 60021318	E	Based on	EP 1077262
ES 2247990	T3	Based on	EP 1077262
DE 60021318	T2	Based on	EP 1077262

PRIORITY APPLN. INFO: EP 1999-114811 19990729
AN 2001-246712 [26] WPIDS
CR 2001-204356 [21]
AB EP 1077262 A UPAB: 20060602

NOVELTY - Production of a naturally folded eukaryotic polypeptide (I) containing two or several cysteines linked by disulfide bridges comprises co-expression and secretion into the periplasm (P) of a molecular chaperone (II).

DETAILED DESCRIPTION - Production of (I) containing two or several cysteines linked by disulfide bridges comprises:

(1) culturing prokaryotic cells containing an expression vector which contains a signal sequence (III) and encodes (I);

(2) secreting (I) into (P) or the medium; and

(3) cleavage of (III) and isolation of (I).

A nucleic acid coding (II) is additionally expressed and is secreted into (P) with the proviso that cultivation is performed without the presence of arginine or a compound of the general formula:

R2-CO-NRR1

R and R1 = H, (un)saturated, (un)branched, chain 1-4C alkyl

R2 = H, NHR1, (un)saturated, (un)branched, chain 1-3C alkyl

USE - For producing a naturally folded eukaryotic polypeptide such as an antibody, antibody fragment, interferon, protein hormone or a protease containing two or several cysteines linked by disulfide bridges (claimed). In particular, the process is useful for the production of proteins which contain more than 5 disulfide bridges in the native state e.g. a recombinant plasminogen activator.

ADVANTAGE - The process for the production of water-soluble naturally folded eukaryotic polypeptides after expression in prokaryotes can be carried out in a simple manner which does not require a laborious in vitro after treatment such as dissolution of inclusion bodies, reduction and renaturation. The simultaneous co-overexpression of (II) in the periplasm enables the yield of functional protein to be increased about 5-10 fold compared to controls.

Dwg.0/6

L12 ANSWER 21 OF 23 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

ACCESSION NUMBER: 2001-204356 [21] WPIDS

CROSS REFERENCE: 2001-246712 [26]

DOC. NO. CPI: C2001-060859

TITLE: Preparation of naturally folded eukaryotic proteins, e.g. antibodies, by simultaneous expression of a chaperone protein, allows simple recovery from periplasm or medium.

DERWENT CLASS: B04 D16

INVENTOR(S): AMBROSIUS, D; RUDOLPH, R; SCHAFFNER, J; SCHWARZ, E; SCHAEFFNER, J

PATENT ASSIGNEE(S): (HOFF) HOFFMANN LA ROCHE & CO AG F; (HOFF) HOFFMANN LA ROCHE INC

COUNTRY COUNT: 30

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1077263	A1	20010221	(200121)*	GE	36
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2001061487	A	20010313	(200124)		25
CN 1283702	A	20010214	(200130)		
KR 2001049895	A	20010615	(200171)		
US 6455279	B1	20020924	(200266)		
KR 378325	B	20030329	(200353)		
MX 2000007339	A1	20020801	(200366)		
EP 1077262	B1	20050720	(200547)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1077263	A1	EP 1999-114811	19990729
JP 2001061487	A	JP 2000-231804	20000731
CN 1283702	A	CN 2000-121500	20000726
KR 2001049895	A	KR 2000-43241	20000727
US 6455279	B1	US 2000-618869	20000719
KR 378325	B	KR 2000-43241	20000727
MX 2000007339	A1	MX 2000-7339	20000727
EP 1077262	B1	EP 2000-115839	20000724

FILING DETAILS:

PATENT NO	KIND	PATENT NO
KR 378325	B Previous Publ.	KR 2001049895

PRIORITY APPLN. INFO: EP 1999-114811 19990729
AN 2001-204356 [21] WPIDS
CR 2001-246712 [26]
AB EP 1077263 A UPAB: 20050725
NOVELTY - Preparing a naturally folded eukaryotic polypeptide (I) that contains two or more disulfide-bridged Cys residues by culturing prokaryotic cells that contain an expression vector for (I) including a prokaryotic signal sequence at its N-terminus and a nucleic acid (II) that secretes a chaperone protein (III) into the periplasm.

DETAILED DESCRIPTION - (I) is secreted into the periplasm or medium; the signal peptide is then cleaved and (I) isolated from the periplasm or medium.

USE - The method is used for production of antibody, interferon, protein hormone or protease.

ADVANTAGE - Expression of (III) increases the yield of (I). The method is simple and eliminates time-consuming in vitro processing operations such as dissolution of inclusion bodies, reduction and refolding. (III) protects (I) against agglomeration and promotes their natural conformation.

Dwg.0/7

L12 ANSWER 22 OF 23 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2001-033777 [05] WPIDS
CROSS REFERENCE: 2000-674185 [66]
DOC. NO. CPI: C2001-010405
TITLE: Producing water-soluble, naturally folded, and secreted eukaryotic polypeptide, involves culturing prokaryotic cells containing an expression vector encoding the polypeptide in the presence of arginine or a specific compound.
DERWENT CLASS: B04 D16
INVENTOR(S): AMBROSIUS, D; RUDOLPH, R; SCHAEFFNER, J; SCHWARZ, E
PATENT ASSIGNEE(S): (HOFF) HOFFMANN LA ROCHE & CO AG F
COUNTRY COUNT: 25
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 1054063	A2	20001122	(200105)*	EN	35
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
EP 1054063	B1	20040915	(200460)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

DE 60013689 E 20041021 (200469)
ES 2228331 T3 20050416 (200528)
DE 60013689 T2 20050929 (200568)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 1054063	A2	EP 2000-108505	20000419
EP 1054063	B1	EP 2000-108505	20000419
DE 60013689	E	DE 2000-00013689	20000419
		EP 2000-108505	20000419
ES 2228331	T3	EP 2000-108505	20000419
DE 60013689	T2	DE 2000-00013689	20000419
		EP 2000-108505	20000419

FILING DETAILS:

PATENT NO	KIND	PATENT NO
DE 60013689	E Based on	EP 1054063
ES 2228331	T3 Based on	EP 1054063
DE 60013689	T2 Based on	EP 1054063

PRIORITY APPLN. INFO: EP 1999-107412 19990426
AN 2001-033777 [05] WPIDS
CR 2000-674185 [66]
AB EP 1054063 A UPAB: 20051024
NOVELTY - Producing water-soluble, naturally folded eukaryotic polypeptide (I), involves culturing prokaryotic cells containing an expression vector encoding (I) having N-terminal prokaryotic signal sequences, in the presence of arginine or a compound (S1), so that (I) is secreted into the periplasm or the medium, cleaving the signal sequence and isolating the polypeptide from the periplasm or medium.

DETAILED DESCRIPTION - Producing (I) which contains two or more cysteines linked by disulfide bridges, involves culturing prokaryotic cells containing an expression vector encoding (I) containing a prokaryotic signal sequence at the N-terminus, in the presence of arginine or a compound of general formula (S1) R2-CO-NRR-1, where

R and R1 = hydrogen, or saturated or unsaturated branched or unbranched C1-C4 alkyl chain; and

R2 = hydrogen, NHR1, or saturated or unsaturated branched or unbranched C1-C3 alkyl chain

under conditions that (I) is secreted into the periplasm or the medium, cleaving the signal sequence and isolating the polypeptide from the periplasm or medium.

USE - The method is useful for producing water-soluble, naturally folded eukaryotic polypeptide such as an antibody, antibody fragment, interferon, protein hormone or a protease (claimed).

ADVANTAGE - The method recombinantly produces a high yield of polypeptides in prokaryotes. The method can be carried out in a simple manner and does not require a laborious in vitro after-treatment such as solubilization, reduction and renaturation of inclusion bodies, reduction and renaturation.

Dwg.0/8

L12 ANSWER 23 OF 23 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2000-376561 [32] WPIDS
CROSS REFERENCE: 2000-320297 [28]
DOC. NO. CPI: C2000-113963

TITLE: High yield expression of recombinant protein, useful e.g. for producing antitumor immunotoxins, by growing host cells under stress conditions in presence of stabilizing solutes.
DERWENT CLASS: B04 D16
INVENTOR(S): BARTH, S; ENGERT, A; GALINSKI, E; HUHN, M; LOUIS, P
PATENT ASSIGNEE(S): (BART-I) BARTH S; (BITO-N) BITOP GES BIOTECHNISCHE OPTIMIERUNG MBH
COUNTRY COUNT: 22
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000028050	A1	20000518	(200032)*	GE	48
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: CA JP US					
EP 1127141	A1	20010829	(200150)	GE	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000028050	A1	WO 1999-EP8025	19991022
EP 1127141	A1	EP 1999-953921	19991022
		WO 1999-EP8025	19991022

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 1127141	A1 Based on	WO 2000028050

PRIORITY APPLN. INFO: EP 1998-121243 19981107; DE 1998-19851408 19981107
AN 2000-376561 [32] WPIDS
CR 2000-320297 [28]
AB WO 200028050 A UPAB: 20010905
NOVELTY - Expression and recovery of recombinant proteins (I) by culturing transformed organisms comprises stressing the organisms by altering their environmental conditions and trigerring production of (I) with an inducer, in presence of compatible solutes.

ACTIVITY - Antitumor.

MECHANISM OF ACTION - Stress conditions induce formation of chaperone proteins.

USE - The method is especially used to produce immunotoxins that can kill cancer cells selectively, or chimeric proteins with several different binding functions, but more generally may be used to produce any protein, e.g. enzyme, antibody fragment, peptide hormone or cytokine.

ADVANTAGE - Application of stress condiitons improves the yield of (I) by:

(i) increasing expression;

(ii) preventing formation of inclusion bodies;

(iii) increasing the proportion of correctly folded material;

(iv) reducing contamination by foreign proteins; and/or

(v) reducing protease-induced decomposition.

The compatible solutes act as stabilizers.

Dwg.0/14

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=> e pluckthun?/au
E1            1     PLUCKTHUN K H/AU
E2           19     PLUCKTHUN M/AU
E3            0 --> PLUCKTHUN?/AU
E4            1     PLUCKTHYN M/AU
E5            2     PLUCKTUM M/AU
E6            1     PLUCKTUN A/AU
E7            1     PLUCLENNICZAK GRAZYNA/AU
E8            7     PLUCNAR B/AU
E9           24     PLUCNAR B J/AU
E10           1     PLUCNAR BOHUMIL/AU
E11           2     PLUCNAR R/AU
E12           1     PLUCONSKI P/AU

=> e pluckthun a?/au
E1            1     PLUCKTHUN/AU
E2          689     PLUCKTHUN A/AU
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E3           0 --> PLUCKTHUN A?/AU
E4         306     PLUCKTHUN ANDREAS/AU
E5           1     PLUCKTHUN ANDREAS GEORG/AU
E6           1     PLUCKTHUN C/AU
E7          27     PLUCKTHUN H/AU
E8          12     PLUCKTHUN HANS/AU
E9           2     PLUCKTHUN J/AU
E10          1     PLUCKTHUN JOHANNES/AU
E11          1     PLUCKTHUN K H/AU
E12         19     PLUCKTHUN M/AU

=> e2 or e4 or e5
L13        996 "PLUCKTHUN A"/AU OR "PLUCKTHUN ANDREAS"/AU OR "PLUCKTHUN ANDREAS
               GEORG"/AU

=> periplas? and l13
L14        105 PERIPLAS?. AND L13

=> fold? and l14
L15         72 FOLD? AND L14

=> dup rem l15
PROCESSING COMPLETED FOR L15
L16         25 DUP REM L15 (47 DUPLICATES REMOVED)

=> d ibib abs l16 1-25

L16  ANSWER 1 OF 25     MEDLINE on STN
ACCESSION NUMBER:   2006418961     IN-PROCESS
DOCUMENT NUMBER:    PubMed ID: 16823375
TITLE:              Signal sequences directing cotranslational translocation
                    expand the range of proteins amenable to phage display.
AUTHOR:             Steiner Daniel; Forrer Patrik; Stumpp Michael T;
                    Pluckthun Andreas
CORPORATE SOURCE:   Department of Biochemistry, University of Zurich,
                    Winterthurerstrasse 190, 8057 Zurich, Switzerland.
SOURCE:             Nature biotechnology, (2006 Jul) Vol. 24, No. 7, pp.
                    823-31.  Electronic Publication: 2006-07-02.
                    Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED;
                    Priority Journals
ENTRY DATE:         Entered STN: 15 Jul 2006
                    Last Updated on STN: 15 Jul 2006
AB   Even proteins that fold well in bacteria are frequently
     displayed poorly on filamentous phages.  Low protein presentation on phage
     might be caused by premature cytoplasmic folding, leading to
     inefficient translocation into the periplasm.  As translocation
     is an intermediate step in phage assembly, we tested the display levels of
     a range of proteins using different translocation pathways by employing
     different signal sequences.  Directing proteins to the cotranslational
     signal recognition particle (SRP) translocation pathway resulted in much
     higher display levels than directing them to the conventional
     post-translational Sec translocation pathway.  For example, the display
     levels of designed ankyrin-repeat proteins (DARPins) were improved up to
     700-fold by simply exchanging Sec- for SRP-dependent signal
     sequences.  In model experiments this exchange of signal sequences
     improved phage display from tenfold enrichment to >1,000-fold
     enrichment per phage display selection round.  We named this method 'SRP
     phage display' and envision broad applicability, especially when
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displaying cDNA libraries or very stable and fast-folding proteins from libraries of alternative scaffolds.

L16 ANSWER 2 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:477157 CAPLUS
DOCUMENT NUMBER: 135:287010
TITLE: Miniantibodies
AUTHOR(S): Lindner, Peter; Pluckthun, Andreas
CORPORATE SOURCE: Universitat Zurich, Biochemisches Institut, Zurich, 8057, Switz.
SOURCE: Antibody Engineering (2001), 637-647. Editor(s): Kontermann, Roland; Duebel, Stefan. Springer-Verlag: Berlin, Germany.
CODEN: 69BLB8
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review with many refs. on the generation of artificial multivalent or multispecific recombinant antibody fragments, called miniantibodies. The basic element of all miniantibody constructs is a fusion of a scFv fragment to an oligomerizing element, giving the partners enough steric freedom to fold individually. This leads to dimeric or tetrameric miniantibodies, depending on the oligomerization motif chosen. The miniantibodies are expressed in the periplasm to allow the disulfide formation in the scFv part. This requires that the chosen oligomerization modules are compatible with periplasmic folding.
REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 3 OF 25 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2001372738 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11428902
TITLE: High enzymatic activity and chaperone function are mechanistically related features of the dimeric E. coli peptidyl-prolyl-isomerase FkpA.
AUTHOR: Ramm K; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstr. 190, Zurich, CH-8057, Switzerland.
SOURCE: Journal of molecular biology, (2001 Jul 6) Vol. 310, No. 2, pp. 485-98.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 30 Jul 2001
Last Updated on STN: 30 Jul 2001
Entered Medline: 26 Jul 2001
AB We have recently described the existence of a chaperone activity for the dimeric peptidyl-prolyl cis/trans isomerase FkpA from the periplasm of Escherichia coli that is independent of its isomerase activity. We have now investigated the molecular mechanism of these two activities in vitro in greater detail. The isomerase activity with a protein substrate (RNaseT1) is characterized by a 100-fold higher k(cat)/K(M) value than with a short tetrapeptide substrate. This enhanced activity with a protein is due to an increased affinity towards the protein substrate mediated by a polypeptide-binding site that is distinct from the active site. The chaperone activity is also mediated by interaction of folding and unfolding intermediates with a binding site that is most likely identical to the polypeptide-binding site which enhances catalysis. Both activities are thus mechanistically

related, being based on the transient interaction with this high-affinity polypeptide-binding site. Only the isomerase activity, but not the chaperone activity, with the substrate citrate synthase can be inhibited by FK520. Experiments with the isolated domains of FkpA imply that both the isomerase and the chaperone site are located on the highly conserved FKBP domain. The additional amino-terminal domain mediates the dimerization and thus places the two active sites of the FKBP domains in juxtaposition, such that they can simultaneously interact with a protein, and this is required for full catalytic activity.

L16 ANSWER 4 OF 25 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2000287592 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10748201
TITLE: The periplasmic Escherichia coli peptidylprolyl cis,trans-isomerase FkpA. II. Isomerase-independent chaperone activity in vitro.
AUTHOR: Ramm K; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
SOURCE: The Journal of biological chemistry, (2000 Jun 2) Vol. 275, No. 22, pp. 17106-13.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20 Jul 2000
Last Updated on STN: 20 Jul 2000
Entered Medline: 11 Jul 2000

AB We recently identified FkpA by selecting for the increased yield of antibody single-chain Fv (scFv) fragments in phage display, even of those not containing cis-prolines. We have now investigated the properties of FkpA in vitro. The peptidylprolyl cis-trans-isomerase activity of FkpA was found to be among the highest of any such enzyme with a protein substrate, yet FkpA is not able to enhance the proline-limited refolding rate of the disulfide-free hu4D5-8 scFv fragment, probably due to inaccessibility of Pro-L95. Nevertheless, the yield of the soluble and functional scFv fragment was dramatically increased in vitro in the presence of FkpA. Similar effects were observed for an scFv fragment devoid of cis-prolines. We are thus forced to conclude that the observed folding-assisting function is independent of the isomerase activity of the protein. The beneficial effect of FkpA was found to be due to two components. First, FkpA interacts with early folding intermediates, thus preventing their aggregation. Additionally, it has the ability to reactivate inactive protein, possibly also by binding to a partially unfolded species that may exist in equilibrium with the aggregated form, which may thus be released on a productive pathway. These in vitro measurements therefore fully reflect the in vivo results from periplasmic overexpression of FkpA.

L16 ANSWER 5 OF 25 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000287591 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10748200
TITLE: The periplasmic Escherichia coli peptidylprolyl cis,trans-isomerase FkpA. I. Increased functional expression of antibody fragments with and without cis-prolines.
AUTHOR: Bothmann H; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.

SOURCE: The Journal of biological chemistry, (2000 Jun 2) Vol. 275, No. 22, pp. 17100-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20 Jul 2000
Last Updated on STN: 20 Jul 2000
Entered Medline: 11 Jul 2000

AB The production of recombinant proteins in the periplasm of Escherichia coli can be limited by folding problems, leading to periplasmic aggregates. We used a selection system for periplasmic chaperones based on the coexpression of an E. coli library with a poorly expressing antibody single-chain Fv (scFv) fragment displayed on filamentous phage (Bothmann, H., and Pluckthun, A. (1998) Nature Biotechnol. 16, 376-380). By selection for a functional antibody, the protein Skp had been enriched previously and shown to improve periplasmic expression of a wide range of scFv fragments. This selection strategy was now repeated with a library constructed from the genomic DNA of an skp-deficient strain, leading to enrichment of the periplasmic peptidylprolyl cis,trans-isomerase (PPIase) FkpA. Coexpression of FkpA increased the amount of fusion protein displayed on the phage and dramatically improved functional periplasmic expression even of scFv fragments not containing cis-prolines. In contrast, the coexpression of the periplasmic PPIases PpiA and SurA showed no increase in the functional scFv fragment level in the periplasm or displayed on phage. Together with the in vitro data in the accompanying paper (Ramm, K., and Pluckthun, A. (2000) J. Biol. Chemical 275, 17106-17113), we conclude that the effect of FkpA is independent of its PPIase activity.

L16 ANSWER 6 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2000123999 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10656818
TITLE: Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides.
AUTHOR: Knappik A; Ge L; Honegger A; Pack P; Fischer M; Wellnhofer G; Hoess A; Wolle J; Pluckthun A; Virnekas B
CORPORATE SOURCE: MorphoSys AG, Lena-Christ-Str. 48, Martinsried/Munich, 82152, Germany.. knappik@morphosys.de
SOURCE: Journal of molecular biology, (2000 Feb 11) Vol. 296, No. 1, pp. 57-86.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1DGX; PDB-1DH4; PDB-1DH5; PDB-1DH6; PDB-1DH7; PDB-1DH8; PDB-1DH9; PDB-1DHA; PDB-1DHO; PDB-1DHQ; PDB-1DHU; PDB-1DHV; PDB-1DHW; PDB-1DHZ
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 27 Mar 2000
Last Updated on STN: 27 Mar 2000
Entered Medline: 14 Mar 2000

AB By analyzing the human antibody repertoire in terms of structure, amino acid sequence diversity and germline usage, we found that seven V(H) and seven V(L) (four Vkappa and three Vlambda) germline families cover more than 95 % of the human antibody diversity used. A consensus sequence was derived for each family and optimized for expression in Escherichia coli.

In order to make all six complementarity determining regions (CDRs) accessible for diversification, the synthetic genes were designed to be modular and mutually compatible by introducing unique restriction endonuclease sites flanking the CDRs. Molecular modeling verified that all canonical classes were present. We could show that all master genes are expressed as soluble proteins in the periplasm of E. coli. A first set of antibody phage display libraries totalling 2x10(9) members was created after cloning the genes in all 49 combinations into a phagemid vector, itself devoid of the restriction sites in question. Diversity was created by replacing the V(H) and V(L) CDR3 regions of the master genes by CDR3 library cassettes, generated from mixed trinucleotides and biased towards natural human antibody CDR3 sequences. The sequencing of 257 members of the unselected libraries indicated that the frequency of correct and thus potentially functional sequences was 61 %. Selection experiments against many antigens yielded a diverse set of binders with high affinities. Due to the modular design of all master genes, either single binders or even pools of binders can now be rapidly optimized without knowledge of the particular sequence, using pre-built CDR cassette libraries. The small number of 49 master genes will allow future improvements to be incorporated quickly, and the separation of the frameworks may help in analyzing why nature has evolved these distinct subfamilies of antibody germline genes.

L16 ANSWER 7 OF 25 CAPLUS
ACCESSION NUMBER: 1999:303258 CAPLUS
DOCUMENT NUMBER: 130:307542
TITLE: A bacterial chaperonin increasing the efficiency of transport of proteins into the periplasmic space
INVENTOR(S): Pluckthun, Andreas; Bothmann, Hendrick
PATENT ASSIGNEE(S): Switz.
SOURCE: PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9922010	A1	19990506	WO 1998-EP6755	19981023
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2305631	AA	19990506	CA 1998-2305631	19981023
EP 1025246	A1	20000809	EP 1998-958867	19981023
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001520890	T2	20011106	JP 2000-518101	19981023
US 6630317	B1	20031007	US 2000-564351	20000501
US 2004152103	A1	20040805	US 2003-643083	20030819

PRIORITY APPLN. INFO.:	EP 1997-118457	A	19971023
	WO 1998-EP6755	W	19981023
	US 2000-564351	A3	20000501

AB A bacterial chaperonin that increases the efficiency of transport of proteins into the periplasmic space is identified and a gene encoding it is cloned. The protein may be useful in increasing yields in the manufacture of proteins in bacterial hosts and in the development of phage display libraries. The protein appears to inhibit protein aggregation in the cytoplasm. Methods of identifying these proteins by assaying for improved folding of a reporter moiety are described. The genes

may be used in combination to increase the yields of correctly folded proteins in the periplasm. Chaperonins increasing the efficiency of folding of a poorly-folding single-chain antibody were screened for using a phasmid library that carried the reporter gene. Repeated rounds of selection by panning identified the genes Skp, FkpA, and SlyX.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 8 OF 25 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:488826 SCISEARCH
THE GENUINE ARTICLE: 208AB
TITLE: Filamentous phage infection: crystal structure of g3p in complex with its coreceptor, the C-terminal domain of TolA
AUTHOR: Lubkowski J (Reprint); Hennecke F; Pluckthun A; Wlodawer A
CORPORATE SOURCE: NCI, Macromol Struct Lab, Frederick Canc Res & Dev Ctr, ABL Basic Res Program, Frederick, MD 21702 USA (Reprint); Univ Zurich, Inst Biochem, CH-8057 Zurich, Switzerland
COUNTRY OF AUTHOR: USA; Switzerland
SOURCE: STRUCTURE, (15 JUN 1999) Vol. 7, No. 6, pp. 711-722. ISSN: 0969-2126.
PUBLISHER: CELL PRESS, 1100 MASSACHUSETTES AVE,, CAMBRIDGE, MA 02138 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 64
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Infection of male Escherichia coli cells by filamentous Ff bacteriophages (M13, fd, and f1) involves interaction of the phage minor coat gene 3 protein (g3p) with the bacterial F pilus (primary receptor), and subsequently with the integral membrane protein TolA (coreceptor). G3p consists of three domains (N1, N2, and CT). The N2 domain interacts with the F pilus, whereas the N1 domain - connected to N2 by a flexible glycine-rich linker and tightly interacting with it on the phage - farms a complex with the C-terminal domain of TolA at later stages of the infection process.

Results: The crystal structure of the complex between g3p N1 and TolA D3 was obtained by fusing these domains with a long flexible linker, which was not visible in the structure, indicating its very high disorder and presumably a lack of interference with the formation of the complex. The interface between both domains, corresponding to similar to 1768 Angstrom(2) of buried molecular surface, is clearly defined. Despite the lack of topological similarity between TolA D3 and g3p N2, both domains interact with the same region of the g3p N1 domain. The fold of TolA D3 is not similar to any previously known protein motifs.

Conclusions: The structure of the fusion protein presented here clearly shows that, during the infection process, the g3p N2 domain is displaced by the TolA D3 domain. The folds of g3p N2 and TolA D3 are entirely different, leading to distinctive interdomain contacts observed in their complexes with g3p N1. We can now also explain how the interactions between the g3p N2 domain and the F pilus enable the g3p N1 domain to form a complex with TolA.

L16 ANSWER 9 OF 25 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 1999365429 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10436087
TITLE: The hierarchy of mutations influencing the folding of antibody domains in Escherichia coli.

AUTHOR: Wall J G; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut der Universitat Zurich, Winterthurerstrasse 190,CH-8057 Zurich, Switzerland.
SOURCE: Protein engineering, (1999 Jul) Vol. 12, No. 7, pp. 605-11. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 25 Sep 1999
Last Updated on STN: 25 Sep 1999
Entered Medline: 14 Sep 1999

AB In a systematic study of the periplasmic folding of antibody fragments in Escherichia coli, we have analysed the expression of an aggregation-prone and previously non-functional anti-phosphorylcholine antibody, T15, as a model system and converted it to a functional molecule. Introduction of heavy chain framework mutations previously found to improve the folding of a related antibody led to improved folding of T15 fragments and improved physiology of the host E.coli cells. Manipulation of the complementarity determining regions (CDR) of the framework-mutated forms of T15 further improved folding and bacterial host physiology, but no improvement was seen in the wild type, suggesting the existence of a hierarchy in sequence positions leading to aggregation. Rational mutagenesis of the T15 light chain led to the production of functional T15 fragments for the first time, with increased levels of functional protein produced from V(H) manipulated constructs. We propose that a hierarchical analysis of the primary amino acid sequence, as we have described, provides guidelines on how correctly folding, functional antibodies might be achieved and will allow further delineation of the decisive structural factors and pathways favouring protein aggregation.

L16 ANSWER 10 OF 25 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1999045649 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9826665
TITLE: Ribosome display efficiently selects and evolves high-affinity antibodies in vitro from immune libraries.
AUTHOR: Hanes J; Jermutus L; Weber-Bornhauser S; Bosshard H R; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Nov 24) Vol. 95, No. 24, pp. 14130-5.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 15 Jan 1999
Last Updated on STN: 25 Jan 2002
Entered Medline: 28 Dec 1998

AB Ribosome display was applied for affinity selection of antibody single-chain fragments (scFv) from a diverse library generated from mice immunized with a variant peptide of the transcription factor GCN4 dimerization domain. After three rounds of ribosome display, positive scFvs were isolated and characterized. Several different scFvs were selected, but those in the largest group were closely related to each other and differed in 0 to 5 amino acid residues with respect to their consensus sequence, the likely common progenitor. The best scFv had a

dissociation constant of (4 +/- 1) x 10(-11) M, measured in solution. One amino acid residue in complementarity determining region L1 was found to be responsible for a 65-fold higher affinity than the likely progenitor. It appears that this high-affinity scFv was selected from the mutations occurring during ribosome display in vitro, and that this constitutes an affinity maturation inherent in this method. The in vitro-selected scFvs could be functionally expressed in the Escherichia coli periplasm with good yields or prepared by in vitro refolding. Thus, ribosome display can be a powerful methodology for in vitro library screening and simultaneous sequence evolution.

L16 ANSWER 11 OF 25 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1998409447 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9737871
TITLE: Factors influencing the dimer to monomer transition of an antibody single-chain Fv fragment.
AUTHOR: Arndt K M; Muller K M; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: Biochemistry, (1998 Sep 15) Vol. 37, No. 37, pp. 12918-26.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 29 Oct 1998
Last Updated on STN: 10 Dec 2002
Entered Medline: 20 Oct 1998

AB Antibody single-chain Fv (scFv) fragments are able to form dimers under certain conditions, and the extent of dimerization appears to depend on linker length, antibody sequence, and external factors. We analyzed the factors influencing dimer-monomer equilibrium as well as the rate of interconversion, using the scFv McPC603 as a model system. In this molecule, the stability of the VH-VL interaction can be conveniently varied by adjusting the ionic strength (because of its influence on the hydrophobic effect), by pH (presumably because of the presence of titratable groups in the interface), and by the presence or absence of the antigen phosphorylcholine, which can be rapidly removed due to its very fast off-rate. It was found that the monomer is the thermodynamically stable form with linkers of 15 and 25 amino acids length under all conditions tested (35 microM or less). The dimer is initially formed in periplasmic expression, presumably by domain swapping, and can be trapped by all factors which stabilize the VH-VL interface, such as the presence of the antigen, high ionic strength, and pH below 7.5. Under all other conditions, it converts to the monomer. Predominantly monomer is obtained during in vitro folding. Monomer is stabilized against dimerization at very high concentrations by the same factors which stabilize the VH-VL interaction. These results should be helpful in producing molecules with defined oligomerization states.

L16 ANSWER 12 OF 25 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1998216571 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9555730
TITLE: Selection for a periplasmic factor improving phage display and functional periplasmic expression.
AUTHOR: Bothmann H; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: Nature biotechnology, (1998 Apr) Vol. 16, No. 4, pp. 376-80.
Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 11 Jun 1998
Last Updated on STN: 11 Jun 1998
Entered Medline: 29 May 1998

AB The efficiency of both phage display in Escherichia coli and periplasmic expression of recombinant proteins may be limited by the same periplasmic folding steps. To search for E. coli factors that improve the efficiency of both procedures, a library of E. coli proteins was coexpressed in a phagemid vector that contained a poorly folding single-chain Fv antibody (scFv) fragment fused to g3p. We enriched, by panning for antigen binding, those phagemids in which the amount of displayed scFv is highest. We thus identified the periplasmic protein Skp/OmpH/HlpA as improving phage display of a wide range of scFv fragments. This occurs as a result of an increase in the amount of hybrid protein displayed on the phage. Coexpression of skp also increases the functional yield of scFv fragments when expressed by secretion to the periplasm.

L16 ANSWER 13 OF 25 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1998075928 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9415446
TITLE: Improving in vivo folding and stability of a single-chain Fv antibody fragment by loop grafting.
AUTHOR: Jung S; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut der Universitat Zurich, Switzerland.
SOURCE: Protein engineering, (1997 Aug) Vol. 10, No. 8, pp. 959-66.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 24 Feb 1998
Last Updated on STN: 24 Feb 1998
Entered Medline: 11 Feb 1998

AB The complementary determining regions (CDRs) from the fluorescein-binding antibody 4-4-20, which yields almost no soluble protein in periplasmic expression in Escherichia coli, were transplanted to the framework of the humanized antibody 4D5. The resulting single-chain Fv fragment (scFv) 4D5Flu showed both a dramatic improvement in soluble expression, even at 37 degrees C, and an improved thermodynamic stability. Antigen affinity was maintained upon this engineering by paying attention to crucial framework-CDR contacts. This demonstrates that the use of superior frameworks is a robust strategy to improve the physical properties of scFv fragments. We also report that the grafted version was selected in phage display over several competing variants of the same antibody with identical binding constant but poorer folding or stability properties. The selection required four panning rounds and a temperature of 37 degrees C and we show that the underlying reason for this selection is a higher fraction of phages carrying functional scFv molecules.

L16 ANSWER 14 OF 25 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 97337429 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9194169
TITLE: Disrupting the hydrophobic patches at the antibody variable/constant domain interface: improved in vivo folding and physical characterization of an engineered scFv fragment.

AUTHOR: Nieba L; Honegger A; Krebber C; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: Protein engineering, (1997 Apr) Vol. 10, No. 4, pp. 435-44.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 2 Sep 1997
Last Updated on STN: 2 Sep 1997
Entered Medline: 18 Aug 1997

AB By constructing Fv and single-chain Fv (scFv) fragments of antibodies, the variable domains are taken out of their natural context in the Fab fragment, where they are associated with the constant domains of the light (CL) and heavy chain (CH1). As a consequence, all residues of the former variable/constant domain interface become solvent exposed. In an analysis of 30 non-redundant Fab structures it was found that at the former variable/constant domain interface of the Fv fragment the frequency of exposed hydrophobic residues is much higher than in the rest of the Fv fragment surface. We investigated the importance of these residues for different properties such as folding in vivo and in vitro, thermodynamic stability, solubility of the native protein and antigen affinity. The experimental model system was the scFv fragment of the anti-fluorescein antibody 4-4-20, of which only 2% is native when expressed in the periplasm of Escherichia coli. To improve its in vivo folding, a mutagenesis study of three newly exposed interfacial residues in various combinations was carried out. The replacement of one of the residues (V84D in VH) led to a 25-fold increase of the functional periplasmic expression yield of the scFv fragment of the antibody 4-4-20. With the purified scFv fragment it was shown that the thermodynamic stability and the antigen binding constant are not influenced by these mutations, but the rate of the thermally induced aggregation reaction is decreased. Only a minor effect on the solubility of the native protein was observed, demonstrating that the mutations prevent aggregation during folding and not of the native protein. Since the construction of all scFv fragments leads to the exposure of these residues at the former variable/constant domain interface, this strategy should be generally applicable for improving the in vivo folding of scFv fragments and, by analogy, also the in vivo folding of other engineered protein domains.

L16 ANSWER 15 OF 25 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 95288293 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7770457
TITLE: Engineered turns of a recombinant antibody improve its in vivo folding.
AUTHOR: Knappik A; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: Protein engineering, (1995 Jan) Vol. 8, No. 1, pp. 81-9.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 13 Jul 1995
Last Updated on STN: 6 Feb 1998
Entered Medline: 6 Jul 1995

AB Using recombinant antibodies functionally expressed by secretion to the periplasm in Escherichia coli as a model system, we identified mutations located in turns of the protein which reduce the formation of

aggregates during in vivo folding or which influence cell stability during expression. Unexpectedly, the two effects are based on different mutations and could be separated, but both mutations act synergistically in vivo. Neither mutation increases the thermodynamic stability in vitro. However, the in vivo folding mutation correlates with the yield of oxidative folding in vitro, which is limited by the side reaction of aggregation. The in vivo folding data also correlate with the rate and activation entropy of thermally induced aggregation. This analysis shows that it is possible to engineer improved frameworks for semi-synthetic antibody libraries which may be important in maintaining library diversity. Moreover, limitations in recombinant protein expression can be overcome by single amino acid substitutions.

L16 ANSWER 16 OF 25 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1994:531435 BIOSIS
DOCUMENT NUMBER: PREV199497544435
TITLE: An improved affinity tag based on the FLAG peptide for the detection and purification of recombinant antibody fragments.
AUTHOR(S): Knappik, Achim; Pluckthun, Andreas [Reprint author]
CORPORATE SOURCE: Dep. Biochem., Univ. Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland
SOURCE: Biotechniques, (1994) Vol. 17, No. 4, pp. 754-761. CODEN: BTNQDO. ISSN: 0736-6205.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Dec 1994
Last Updated on STN: 15 Dec 1994

AB The commercially available monoclonal antibodies M1 and M2 were raised against and bind the FLAG sequence DYKDDDDK with high specificity. Using the calcium-dependent MI antibody and the FLAG tag attached to the N terminus of various fragments of the antibody McPC603 expressed in Escherichia coli, we found that the MI antibody binds with almost the same affinity to a much shorter version of this sequence (DYKD). Since most antibody light chains start with an aspartate, the addition of only three additional amino acids to the N terminus is sufficient to detect and quantify the expressed antibody fragments using standard immunological methods. Similarly, the heavy chain can be detected specifically with the sequence DYKD, which requires four additional amino acids since most heavy chains do not start with Asp. The signal sequence of both chains that is necessary for the transport of the chains to the periplasm of E. coli is processed correctly. Furthermore, we investigated the influence of the amino acid at the fifth position of the FLAG sequence on the binding affinity of the M1 antibody and found that a glutamate at this position increased the sensitivity in Western blots six-fold over the original long FLAG sequence containing an aspartate residue at this position. Together the improved FLAG is a versatile tool for both sensitive detection and one-step purification of recombinant proteins.

L16 ANSWER 17 OF 25 MEDLINE on STN DUPLICATE 11

ACCESSION NUMBER: 94328921 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8052121
TITLE: Protein folding in the periplasm of Escherichia coli.
AUTHOR: Wulfing C; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: Molecular microbiology, (1994 Jun) Vol. 12, No. 5, pp. 685-92. Ref: 96
Journal code: 8712028. ISSN: 0950-382X.

PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 14 Sep 1994
Last Updated on STN: 14 Sep 1994
Entered Medline: 6 Sep 1994

AB With the discovery of molecular chaperones and the development of heterologous gene expression techniques, protein folding in bacteria has come into focus as a potentially limiting factor in expression and as a topic of interest in its own right. Many proteins of importance in biotechnology contain disulphide bonds, which form in the Escherichia coli periplasm, but most work on protein folding in the periplasm of E. coli is very recent and is often speculative. This MicroReview gives a short overview of the possible fates of a periplasmic protein from the moment it is translocated, as well as of the E. coli proteins involved in this process. After an introduction to the specific physiological situation in the periplasm of E. coli, we discuss the proteins that might help other proteins to obtain their correctly folded conformation--disulphide isomerase, rotamase, parts of the translocation apparatus and putative periplasmic chaperones--and briefly cover the guided assembly of multi-subunit structures. Finally, our MicroReview turns to the fate of misfolded proteins: degradation by periplasmic proteases and aggregation phenomena.

L16 ANSWER 18 OF 25 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 95018232 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7932722
TITLE: Correctly folded T-cell receptor fragments in the periplasm of Escherichia coli. Influence of folding catalysts.
AUTHOR: Wulfing C; Pluckthun A
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Protein Engineering Group, Martinsried, Germany.
SOURCE: Journal of molecular biology, (1994 Oct 7) Vol. 242, No. 5, pp. 655-69.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 22 Dec 1994
Last Updated on STN: 22 Dec 1994
Entered Medline: 27 Oct 1994

AB The T-cell receptor is the central recognition molecule in cellular immunity. Its extracellular domains are homologous with and thought to be structurally similar to an antibody Fab fragment. Despite the biological importance of the TCR and the ease of bacterial expression of antibody fragments, there are only few reports of TCR-fragment expression in E. coli. In order to understand the difficulties of expressing correctly folded TCR fragments in E. coli, we have characterized the expression behavior of single-chain Fv analogs of three different TCRs (scTCR). All of them can be folded into the correct conformation in the periplasm of E. coli, yet the extent of correct folding varies greatly. In order to overcome the folding problems of some of the scTCRs, we have developed a system with enhanced in vivo folding capability based on the simultaneous induction of the heat-shock response and over-expression of

the E. coli disulfide isomerase DsbA at low temperature. We present a model describing the folding of the scTCRs in the periplasm of E. coli and possible points of folding assistance. The role of the periplasm as an independent folding compartment is emphasized and the existence of a general periplasmic chaperone is postulated. We have also shown that a bivalent scTCR, dimerized in vivo with helix-turn-helix modules, can be expressed in a correctly folded form.

L16 ANSWER 19 OF 25 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 93207728 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7763488
TITLE: The effect of folding catalysts on the in vivo folding process of different antibody fragments expressed in Escherichia coli.
AUTHOR: Knappik A; Krebber C; Pluckthun A
CORPORATE SOURCE: Max-Planck-Institut fur Biochemie, Protein Engineering Group, Martinsried, Fed. Rep. Germany.
SOURCE: Bio/technology (Nature Publishing Company), (1993 Jan) Vol. 11, No. 1, pp. 77-83.
Journal code: 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 9 Aug 1995
Last Updated on STN: 6 Feb 1998
Entered Medline: 29 Apr 1993
AB The Fv and Fab fragment and both orientations of the single-chain Fv fragment (VH-linker-VL and VL-linker-VH) of an antibody can be expressed in functional form in the periplasm of Escherichia coli, but the yield of these correctly assembled proteins is limited by the periplasmic folding process. While the periplasmic E. coli disulfide isomerase DsbA is required for this assembly, its functional over-expression does not significantly change the folding limit. Similarly, the functionally over-expressed E. coli proline cis-trans isomerase does not change the amount of all but one of the antibody fragments, not even if DsbA is over-expressed as well. Therefore, aggregation steps in the periplasm appear to compete with periplasmic folding, and they may occur before disulfide formation and/or proline cis-trans isomerization takes place and be independent of their extent.

L16 ANSWER 20 OF 25 MEDLINE on STN DUPLICATE 14
ACCESSION NUMBER: 92144530 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1736986
TITLE: The disulfide bonds in antibody variable domains: effects on stability, folding in vitro, and functional expression in Escherichia coli.
AUTHOR: Glockshuber R; Schmidt T; Pluckthun A
CORPORATE SOURCE: Genzentrum, Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, Germany.
SOURCE: Biochemistry, (1992 Feb 11) Vol. 31, No. 5, pp. 1270-9.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199203
ENTRY DATE: Entered STN: 5 Apr 1992
Last Updated on STN: 3 Mar 2000

Entered Medline: 16 Mar 1992

AB The formation of the disulfide bonds in the variable domains VH and VL of the antibody McPC603 was found to be essential for the stability of all antigen binding fragments investigated. Exposure of the Fv fragment to reducing conditions in vitro resulted in irreversible denaturation of both VH and VL. In vitro refolding of the reduced Fv fragment was only possible when the disulfide bonds were allowed to form under oxidizing conditions. The analysis of a series of mutants of the Fv fragment, the Fab fragment and the single-chain Fv fragment, all secreted into the periplasm of Escherichia coli, in which each of the cysteine residues of the variable domains was replaced by a series of other amino acids, showed that functional antigen binding fragments required the presence of both the disulfide bond in VH and the one in VL. These results were also used to devise an alternative expression system based on the production of insoluble fusion proteins consisting of truncated beta-galactosidase and antibody domains, enzymatic cleavage, and refolding and assembly in vitro. This strategy should be useful for providing access to unstable antibody domains and fragments.

L16 ANSWER 21 OF 25 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 92285341 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1817261
TITLE: Secretion and in vivo folding of the Fab fragment of the antibody McPC603 in Escherichia coli: influence of disulphides and cis-prolines.
AUTHOR: Skerra A; Pluckthun A
CORPORATE SOURCE: Genzentrum der Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, Germany.
SOURCE: Protein engineering, (1991 Dec) Vol. 4, No. 8, pp. 971-9. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 17 Jul 1992
Last Updated on STN: 17 Jul 1992
Entered Medline: 6 Jul 1992

AB Using the well-characterized antibody McPC603 as a model, we had found that the Fv fragment can be isolated from Escherichia coli as a functional protein in good yields, whereas the amount of the correctly folded Fab fragment of the same antibody produced under identical conditions is significantly lower. In this paper, we analyse the reasons for this difference. We found that a variety of signal sequences function in the secretion of the isolated chains of the Fab fragment or in the co-secretion of both chains in E.coli. The low yield of functional Fab fragment is not caused by inefficient expression or secretion in E.coli, but by inefficient folding and/or assembly in the periplasm. We compared the folding yields for the Fv and the Fab fragment in the periplasm under various conditions. Several diagnostic framework variants were constructed and their folding yields measured. The results show that substitutions affecting cis-proline residues and those affecting various disulphide bonds in the protein are by themselves insufficient to dramatically change the partitioning of the folding pathway to the native structure, and the cause must lie in a facile aggregation of folding intermediates common to all structural variants. However, all structural variants could be obtained in native form, demonstrating the general utility of the secretory expression strategy.

L16 ANSWER 22 OF 25 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 91337413 MEDLINE

DOCUMENT NUMBER: PubMed ID: 1367302
TITLE: The functional expression of antibody Fv fragments in Escherichia coli: improved vectors and a generally applicable purification technique.
AUTHOR: Skerra A; Pfitzinger I; Pluckthun A
CORPORATE SOURCE: Genzentrum, Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, FRG.
SOURCE: Bio/technology (Nature Publishing Company), (1991 Mar) Vol. 9, No. 3, pp. 273-8.
Journal code: 8309273. ISSN: 0733-222X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199109
ENTRY DATE: Entered STN: 9 Aug 1995
Last Updated on STN: 9 Aug 1995
Entered Medline: 20 Sep 1991

AB We have previously demonstrated that the expression of fully functional Fv and Fab fragments in E. coli is possible by the simultaneous secretion of both chains to the periplasm. To increase production levels and facilitate engineering and random mutagenesis, we improved our previous vectors by introducing a resident repressor gene and a filamentous phage origin. We also developed a new purification strategy based on immobilized metal ion chromatography, with which a single-chain Fv fragment can be purified to homogeneity in a single step. We investigated the most efficient tail constructions and found that only a minimal structural change of three additional C-terminal amino acids is necessary. This modification has no deleterious effect on in vivo transport and folding or antigen affinity.

L16 ANSWER 23 OF 25 MEDLINE on STN
ACCESSION NUMBER: 91264723 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2096820
TITLE: Properties of FV and Fab fragments of the antibody McPC603 expressed in E. coli.
AUTHOR: Pluckthun A; Glockshuber R; Skerra A; Stadmuller J
CORPORATE SOURCE: Genzentrum der Universitat Munchen, Germany.
SOURCE: Behring Institute Mitteilungen, (1990 Dec) No. 87, pp. 48-55.
Journal code: 0367532. ISSN: 0301-0457.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 2 Aug 1991
Last Updated on STN: 3 Feb 1997
Entered Medline: 17 Jul 1991

AB The FV and Fab fragments of the phosphorylcholine binding antibody McPC603 were functionally expressed in E. coli. This was achieved by the co-expression and co-secretion of both chains to the periplasm, where correct processing, folding and assembly occurred. Interestingly, the fraction of correctly folded Fab fragment is smaller than that of the Fv fragment in E. coli. The intrinsic hapten binding affinity was shown to be identical for the recombinant FV or Fab fragment, the whole antibody and the Fab fragment obtained by proteolysis from the mouse antibody. Fluorescence and crosslinking analyses showed that the FV fragment dissociates at high dilution, but that it is stabilized by hapten binding. The recombinant FV fragment was shown to have catalytic activity to hydrolyze choline-p-nitrophenyl carbonate and

constitutes therefore a promising model system with which the structural requirements of catalytic antibodies can be studied by altering the protein itself.

L16 ANSWER 24 OF 25 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 89008278 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3049576
TITLE: Membrane-bound beta-lactamase forms in Escherichia coli.
AUTHOR: Pluckthun A; Pfitzinger I
CORPORATE SOURCE: Genzentrum der Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, West Germany.
SOURCE: The Journal of biological chemistry, (1988 Oct 5) Vol. 263, No. 28, pp. 14315-22.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M23206; GENBANK-M23207; GENBANK-M23208; GENBANK-M23209
ENTRY MONTH: 198811
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990
Entered Medline: 7 Nov 1988

AB Frameshift pseudo-revertants of Escherichia coli RTEM beta-lactamase were obtained by a selection procedure, starting from frameshift mutants at the signal-processing site. These pseudo-revertant proteins, which have a totally altered COOH-terminal part of the signal sequence, are attached to the outer face of the inner membrane. The mutant proteins are enzymatically active in vitro and in vivo, and the membrane localization has no deleterious effect on cell growth. We conclude that initiation of transport across the membrane does not require the COOH-terminal part of the signal, but this part of the sequence determines whether the protein is released to the periplasm either with or without cleavage of the signal, or whether the protein remains anchored to the membrane. Mutants with two signals in series were used to show that a truncated signal is not refractory to transport per se. If neither signal contains a functional cleavage site, the protein is at least partially found on the outer face of the inner membrane. If both signals contain functional cleavage sites, both are removed and the protein is released to the periplasm. If only the first signal contains a cleavage site, a longer fusion protein is transported and released. The results presented here show that a pre-beta-lactamase-like protein can fold properly even as a membrane-bound species.

L16 ANSWER 25 OF 25 MEDLINE on STN
ACCESSION NUMBER: 88218715 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3285470
TITLE: Assembly of a functional immunoglobulin Fv fragment in Escherichia coli.
AUTHOR: Skerra A; Pluckthun A
CORPORATE SOURCE: Genzentrum der Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, FRG.
SOURCE: Science, (1988 May 20) Vol. 240, No. 4855, pp. 1038-41.
Journal code: 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198806
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990

Entered Medline: 23 Jun 1988

AB An expression system was developed that allows the production of a completely functional antigen-binding fragment of an antibody in Escherichia coli. The variable domains of the phosphorylcholine-binding antibody McPC603 were secreted together into the periplasmic space, where protein folding as well as heterodimer association occurred correctly. Thus, the assembly pathway for the Fv fragment in E. coli is similar to that of a whole antibody in the eukaryotic cell. The Fv fragment of McPC603 was purified to homogeneity with an antigen-affinity column in a single step. The correct processing of both signal sequences was confirmed by amino-terminal protein sequencing. The functionality of the recombinant Fv fragment was demonstrated by equilibrium dialysis. These experiments showed that the affinity constant of the Fv fragment is identical to that of the native antibody McPC603, that there is one binding site for phosphorylcholine in the Fv fragment, and that there is no inactive protein in the preparation. This expression system should facilitate future protein engineering experiments on antibodies.

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